Pathol. Oncol. Res. (2009) 15:651–658
DOI 10.1007/s12253-009-9167-8

ORIGINAL PAPER

Clinicopathologic and Molecular Features of Epidermal Growth Factor Receptor T790M Mutation and c-MET Amplification in Tyrosine Kinase Inhibitor-resistant Chinese Non-small Cell Lung Cancer

Hua-Jun Chen · Tony S. Mok · Zhi-Hong Chen · Ai-Lin Guo · Xu-Chao Zhang · Jian Su · Yi-Long Wu

Received: 24 February 2009 / Accepted: 6 April 2009 / Published online: 21 April 2009
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Abstract To investigate the clinicopathologic and molecular features of the T790M mutation and c-MET amplification in a cohort of Chinese non-small cell lung cancer (NSCLC) patients resistant to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs). EGFR TKI-resistant NSCLC patients (n=29) and corresponding tumor specimens, and 53 samples of postoperative TKI-naïve NSCLC patients were collected. EGFR exon 19, 20, and 21 mutations were analyzed. And c-MET gene copy number was determined. The EGFR T790M mutation in exon 20 was not detected in the population of 53 TKI-naïve patients, but found in 48.3% (14/29) of the enrolled TKI-resistant patients. c-MET was amplified in 3.8% (2/53) of the TKI-naïve NSCLC patients and highly amplified in 17.2% (5/29) of the cohort. Most of T790M mutations were frequently associated with non-smoker, adenocarcinoma and EGFR activating mutations. Three male patients with T790M mutation occurred with wild-type EGFR, and were resistant to the treatments following TKI resistance. Features of c-MET amplification in TKI-naïve patients were indistinguishable from TKI-resistant patients. In the group of wild-type EGFR, patients with T790M mutation had median progression free survival (PFS) and overall survival (OS) as 9.6 months and 12.6 months, respectively; whereas the median PFS and OS of c-MET amplified patients was 4.1 months and 8.0 months, respectively. These results suggest that EGFR T790M mutation and c-MET amplification can occur in TKI-resistant NSCLC with wild-type EGFR, and these genetic defects might be related to different survival outcome. c-MET amplification in TKI-naïve or -resistant patients might share similarities in clinicopathologic features.

Keywords c-MET · Epidermal growth factor receptor · Non-small cell lung cancer, Resistance · T790M

Abbreviations

(ARMS)	amplification refractory mutation system
(EGFR)	epidermal growth factor receptor
(NSCLC)	non-small cell lung cancer
(OS)	overall survival
(PFS)	progression free survival
(TKI)	tyrosine kinase inhibitor

The data were presented in part at the 2008 ASCO annual meeting (Abstract-No. 8107).

H.-J. Chen
Cancer Center, Sun Yat-Sen University,
Guangzhou, China

H.-J. Chen · Z.-H. Chen · J. Su · Y.-L. Wu (✉)
Guangdong Lung Cancer Institute, Guangdong General Hospital,
106 Zhongshan Er Road,
Guangzhou, Guangdong 510080, China
e-mail: syylwu@live.cn

T. S. Mok
Department of Clinical Oncology,
Chinese University of Hong Kong,
Shatin, New Territories,
Hong Kong, China

A.-L. Guo · X.-C. Zhang
Department of Biochip,
Guangdong Academy of Medical Sciences,
Guangzhou, China

Introduction

The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib are effective in the treatment of non-small cell lung cancer (NSCLC), but resistance develops eventually in patients after an initial response. The mechanisms responsible for the resistance are not fully understood.

The most common EGFR mutation associated with treatment resistance is due to the occurrence of a secondary mutation in the EGFR kinase domain [1, 2]. Specifically, the mutation involves a C to T transversion at nucleotide 2369 in exon 20, which results in substitution of methionine for threonine at position 790 (T790M). Substitution with this bulky methionine residue had been suggested to cause resistance by sterically blocking binding of gefitinib or erlotinib [3, 4], but a more recent study indicated that the T790M mutation caused drug resistance by increasing the affinity for ATP [5]. Another mechanism that contributes to EGFR TKI resistance is c-MET amplification, which is detected in about 20% of gefitinib-resistant patients. It has been shown that c-MET caused gefitinib resistance by driving ErbB3 (HER3)-dependent activation of PI3K [6].

In previous *in vitro* studies, the EGFR T790M mutation and amplified c-MET were shown to occur in sensitive EGFR-mutant NSCLC cell lines after exposure to gefitinib. For example, PC-9 and H3255 became resistant to gefitinib and acquired a T790M mutation [7, 8], while in HCC827 cell, c-MET amplification was detected after resistance to gefitinib had developed [6]. All of the above mentioned studies were based on EGFR activating mutations. But 17.2% of EGFR wild-type patients responded to TKIs, which indicated there was a need to concern the mutational status of EGFR and those genetic defects [9]. And, data about features of EGFR T790M mutation and c-MET amplification in EGFR TKI-resistant patients are scarce. Examination of this issue may be helpful in understanding the complexity of EGFR TKI resistance and in choosing appropriate treatments in tumors resistant to EGFR TKIs.

To investigate the clinicopathologic and molecular features of T790M-mutated EGFR and amplified c-MET in Chinese NSCLC, we collected and analyzed a cohort of NSCLC cases resistant to gefitinib or erlotinib.

Materials and Methods

Patients and Tissues

A total of 29 gefitinib- or erlotinib-resistant NSCLC cases were collected from Guangdong General Hospital, Guangzhou, China, between 2004 and 2008. Tumor specimens and corresponding normal lung tissues from 53 postoperative TKI-naïve NSCLC patients were collected to establish the baseline rates of c-MET copy number and the EGFR T790M mutation. All tumor or normal tissues obtained at the time of biopsy or surgical resection were snap frozen in liquid nitrogen and stored at −80°C. Tumors were subtyped histologically according to the World Health Organization (WHO) classification of tumors [10]. For nine TKI-resistant patients, paired tumor specimens were obtained from before TKI treatment and after the development of resistance to gefitinib or erlotinib. The observation period ranged from 5.3 to 53.6 months, with a median follow-up of 24.1 months.

The protocol was approved and monitored by the local institutional review board. All patients provided written informed consent to participation in the study.

Preparation of Genomic DNA

Hematoxylin and eosin-stained sections of frozen specimens were reviewed by a pathologist to identify regions of tumor cells, and laser microdissection (LMD) (Leica AS LMD System; Leica Microsystems Ltd., Milton Keynes, UK) was performed to isolate purified cancer cells for further analysis [11, 12]. Genomic DNA was extracted with the QIAamp DNA micro kit (Qiagen, Courtaboeuf Cedex, France) according to the manufacturer's protocol.

Mutational Analysis

Mutations in three exons (exon 19–21) of the tyrosine kinase (TK) domain of the EGFR gene were detected using a polymerase chain reaction (PCR)-based direct sequencing method adopted from the literature [13, 14]. The PCR primers are shown in Table 1. The first PCR was carried out in a total volume of 25 μL containing 1/25 of the extracted genomic DNA. Other reaction components were as follows: 10 μL of TaqMan Universal PCR MasterMix (Applied Biosystems, Foster City, CA), each PCR primer at 0.4 μM, and an appropriate volume of ultrapure H_2O. PCR was carried out on a MyCycler thermal cycler (Bio-Rad, Hercules, CA). PCR cycling conditions for the three exons were as follows: initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min, with a final extension step at 72°C for 5 min. PCR products were electrophoresed on an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). Sequence variants were determined using the Seqscape software (Applied Biosystems) and confirmed by independent PCR amplification and sequencing in both directions.

Table 1 Primers sequences and annealing temperatures for direct sequencing

Primer	Sequence	Annealing Tm	Product size
19 exon (F)	5′-GCAATATCAGCCTTAGGTGCGGCTC-3′	58°C	372 bp
19 exon (R)	5′-CATAGAAAGTGAACATTTAGGATGTG-3′		
20 exon (F)	5′-CCATGAGTACGTATTTTGAAACTC-3′	58°C	408 bp
20 exon (R)	5′-CATATCCCCATGGCAAACTCTTGC-3′		
21 exon (F)	5′-ATGAACATGACCCTGAATTCGG-3′	58°C	357 bp
21 exon (R)	5′-GCTCACCCAGAATGTCTGGAGA-3′		

T790M Mutation by Scorpions ARMS

We used Scorpions Amplification Refractory Mutation System (DxS, Manchester, UK) [ARMS], which combined the two technologies ARMS and Scorpion, to detect T790M mutation in real-time PCR reactions. All reactions in 20-μL volumes were carried out according to the manufacturer's protocol, containing 5 μL of template DNA (10 ng) or 2.5 μL Standard plus 2.5 μL water or 5 μL water for NTC, 8.5 μL of Control reaction mix or 8.5 μL T790M reaction mix, 10 μL of primer mix and 0.2 μL *Taq* polymerase for control reaction or 0.8 μL *Taq* polymerase for the T790M reaction, 1.3 μL water for control reaction or 0.7 μL water for T790M reaction. Real-time PCR was carried out using Stratagene Mx3005p under the following conditions: initial denaturation at 95°C for 10 min, 40 cycles of 95°C for 30 s, and 61°C for 60 s with fluorescence reading (FAM and HEX). Definition of positive or negative results was decided according to the product's protocol. Analysis of each sample was carried out in duplicate [15, 16].

Quantitative Real-time PCR

The gene copy number of c-MET was assessed by quantitative relative real-time polymerase chain reaction (QPCR). Primers and probes for c-MET were designed according to the sequence in GenBank (Accession No. NM_001127500). Line-1 was used as an endogenous reference gene. The PCR primers and TaqMan probe sequences are available on request.

To produce real-time PCR standards, c-MET and Line-1 were amplified by reverse transcription PCR with gene-specific primers. The amplicons were cloned into the pGEM-T Easy vector (Catalog No. A1360; Promega, Madison WI) and confirmed by sequencing. Purified recombinant DNA was quantified by determining the A_{260} (GeneQuant; Amersham Biosciences, Piscataway, NJ) and then serially diluted in ultrapure H_2O to final concentrations ranging from 10^9 to 10^2 copies/μL. Aliquots of 1 μL of tenfold serially diluted plasmid DNA were used as quantification standards in experimental samples. A new standard curve was run for each real-time PCR.

Real-time PCR was performed in separate 20-μL reaction mixtures for c-MET and Line-1 quantification. The reaction components were as follows: 10 μL of TaqMan Universal PCR MasterMix (Applied Biosystems), each PCR primer at 0.3 μM, and probe at 0.2 μM, and ultrapure H_2O was used to bring the reaction volume to 20 μL. PCR was carried out on with an ABI Prism 7000 Sequence Detection System (Applied Biosystems). PCR cycling conditions for both amplicons were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Each test run included one no-target control. Real-time PCR of the gene was performed in three independent experiments, and the mean ratio value (c-MET/Line-1) was used for quantification. The cut-off value was established as the mean (M)+2 standard deviation (SD) from normal lung tissues of 53 EGFR TKI-naïve patients [17]. A tumor sample was defined as amplification positive if its ratio value was over M+2×SD. DNA samples from NSCLC cell lines HCC827 parental and HCC827 GR6 were used as negative and positive controls, respectively [6].

Statistical Analysis

X^2 or Fisher's exact test was used to compare qualitative data. The latter test was applied to five or fewer observations in a group. Progression free survival (PFS) was the interval from the initiation of gefitinib or erlotinib administration to first appearance of progressive disease or death from any cause. Overall survival (OS) was the interval from the initiation of gefitinib or erlotinib administration to last visit or death. Radiographic response to EGFR TKIs treatment was determined by RECIST [18]. All statistical tests were two-sided, and $P<0.05$ was taken to indicate statistical significance.

Results

Clinicopathologic Characteristics of the Whole Cohort

Characteristics of 53 TKI-naïve NSCLC patients were listed in Table 2. Twenty-nine TKI-resistant advanced

Table 2 Characteristics of 53 EGFR TKI-naive NSCLC

Characteristic	n	%
Age (years)		
Median	58	
Range	33–83	
Gender		
Male	33	62
Female	20	38
Smoking status		
Smoker	31	58
Non-smoker	22	42
Histology		
Adenocarcinoma	39	74
Squamous cell carcinoma	7	13
Large cell carcinoma	3	6
Other types	4	7
TNM staging		
I+II	44	83
IIIA+IIIB	9	17

Table 3 Clinical characteristics of 29 EGFR TKI-resistant NSCLC

Characteristic	n	%
Age (years)		
Median	58	
Range	38–71	
Gender		
Male	18	62
Female	11	38
Smoking status		
Smoker	6	21
Non-smoker	23	79
Histology		
Adenocarcinoma	25	86
Squamous cell carcinoma	2	7
Other types	2	7
First line therapy		
Chemotherapy	21	72
EGFR-TKIs	8	28
Biopsy sites of resistant tumor		
Primary lesions	16	55
Metastatic lesions	13	45

NSCLC patients with a median age of 58 years (range, 38–85) were enrolled in the present study. 79.3% (23/29) of the patients were non-smoker, and 86% (25/29) showed adenocarcinoma histology. The majority of patients received chemotherapy as the first-line regimen, while only 28% (8/29) underwent EGFR TKIs treatment in the first-line setting (Table 3).

Prevalence of EGFR T790M Mutation and c-MET Amplification

The EGFR T790M mutation in exon 20 was not detected in the population of 53 TKI-naïve patients, but 48.3% (14/29) of the enrolled TKI-resistant patients were identified as positive for the T790M mutation. Seven cases were detected by both methods, and the other seven cases were detected by Scorpions ARMS alone. Among 14 T790M mutated patients, 2 also harbored c-MET amplification (Fig. 1). In nine patients with paired specimens, all pre-treatment specimens were negative in T790M mutation, but five post-treatment specimens were identified as T790M positive.

c-MET was amplified in 3.8% (2/53) of the TKI-naïve NSCLC patients according to the established cut-off value (M+2×SD from corresponding normal lung tissues). In the EGFR TKI-resistant NSCLC cohort, c-MET amplification was identified in 17.2% (5/29) of patients with post-treatment tissues (including two patients with concurrent T790M mutation) (Fig. 1 and 2), which was higher than that in EGFR TKI-naïve cases (P=0.045, Fisher's exact test). In nine patients with paired tumor specimens, three showed c-MET amplification in the resistant specimens, but not in the pre-treatment specimens.

Clinicopathologic and Molecular Features in Patients with Different Genetic Defects

In 12 patients with EGFR T790M mutation, Most of them were frequently associated with non-smoker and adenocar-

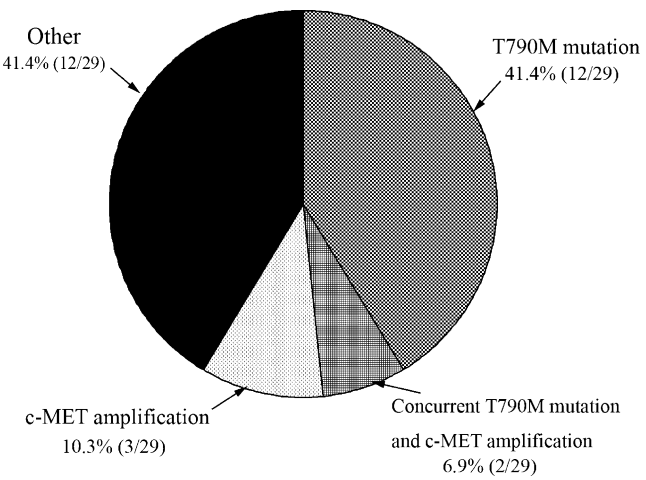

Fig. 1 Prevalence of epidermal growth factor receptor (EGFR) T790M mutation and c-MET amplification in tyrosine kinase inhibitor-resistant Chinese non-small cell lung cancer (NSCLC)

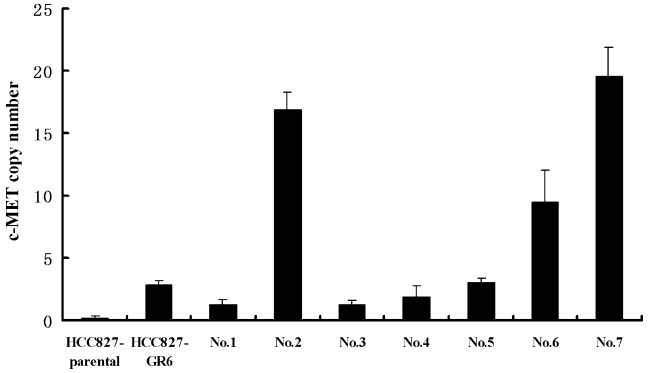

Fig. 2 c-MET relative copy number was determined by quantitative relative real-time polymerase chain reaction (QPCR). HCC827 parental and GR6 were used as negative and positive controls, respectively. No.1 and 2 represented TKI-naïve patients; No.3–7 represented TKI-resistant patients. Error bars indicate standard deviation

cinoma. All patients had no response to treatments following TKI resistance. Nine patients had concurrent EGFR activating mutation, specifically, six cases with in-frame deletions in EGFR 19 exon and three cases with missense mutation in EGFR 21 exon. Notably, three male patients with wild-type EGFR were detected as T790M mutation, and one of them was tested T790M mutation negative in pre-treatment peripheral blood. Median PFS and OS in T790M positive patients with mutated EGFR were 16.9 months and 30.7 months, respectively. T790M positive patients with wild-type EGFR had median PFS and OS as 9.6 months and 12.6 months, respectively (Table 4).

Of two patients detected as concurrent T790M mutation and c-MET amplification, one was female with EGFR activating mutation; the other was male with wild-type EGFR. Both of them were non-smoker and presented adenocarcinoma histology (Table 5).

c-MET amplification in TKI-naïve or -resistant patients shared similar features, including male gender, wild-type EGFR, and had no bias to types of histology. In TKI-resistant patients with amplified c-MET alone, median PFS and OS was 4.1 months and 8.0 months, respectively (Table 6).

Discussion

The use of tyrosine kinase inhibitors to target the EGFR in patients with NSCLC is effective, but limited by the emergence of acquired resistance. As two major mechanisms of drug resistance, the EGFR T790M mutation and c-MET amplification have attracted a great deal of interest in lung cancer research.

About half of NSCLC patients are detected as EGFR T790M positive after the development of resistance to EGFR TKIs [19, 20]. Similarly, EGFR T790M mutation in

Table 4 Characteristics of patients with EGFR T790M mutation

No.	Age	Sex	Smoking	Stage	Histology	Drug	Response[c]	PFS	Site of biopsy	Treatment[d]	Response[e]	OS	Primary mutation
1	59	M	S	IV	AD	Gef.	PR	10.6	Lymph node	WBI	PD	11.6	Wild type
2[a]	45	M	NS	IV	AD	Gef.	SD	4.0	Subcutaneous nodule	BSC	PD	5.9	Wild type
3	69	M	NS	IV	AD	Gef.	PR	14.2	Primary tumor	IP	PD	20.3	Wild type
4	67	F	NS	WetIIIB	AD	Gef.	PR	18.9	Lymph node	Docetaxel	PD	26.2	del E746-A750
5	42	M	NS	IV	AD	Gef.	PR	13.4	Primary tumor	Docetaxel	PD	22.0	del E746-A750
6	53	F	NS	IV	AD	Gef.	PR	17.8	Primary tumor	BSC	PD	18.8	L858R
7[b]	53	F	NS	IV	AD	Gef.	SD	17.7	Primary tumor	Pemetrexed	SD	43.5	del L747-S752
8[b]	85	M	NS	IV	AD	Gef.	SD	22.2	Primary tumor	Pemetrexed	SD	38.1	L858R
9	54	F	NS	IB	AD	Gef.	PR	30.3	Primary tumor	Docetaxel	SD	47.9	L858R
10[b]	60	M	NS	IV	AD	Gef.	SD	4.7	Primary tumor	Docetaxel	SD	12.5	del L747-A750 Ins P
11	50	F	NS	IV	BAC	Gef.	PR	9.2	Primary tumor	TC	SD	18.4	del E746-A750
12	70	F	NS	IIIA	AD	Gef.	SD	17.5	Subcutaneous nodule	Pemetrexed	PD	48.8	del E746-A750

[a] DNA from pre-treatment peripheral blood was detected as T790M mutation negative by both direct sequencing and Scorpions ARMS; [b] Patients with paired specimens; [c] Best response to EGFR TKIs; [d] Treatment following TKI resistance; [e] Response to treatment following TKI resistance

PFS progression free survival (months); *OS* overall survival (months); *M* male; *F* female; *S* smoker; *NS* non-smoker; *AD* adenocarcinoma; *BAC* bronchial alveolar carcinoma; *Gef.* gefitinib; *PR* partial response; *SD* stable disease; *PD* progressive disease; *WBI* whole brain irradiation; *BSC* best supportive care; *IP* irinotecan/cisplatin; *TC* taxol/carboplatin

Table 5 Features of patients with concurrent EGFR T790M mutation and c-MET amplification

No.	Age	Sex	Smoking	Stage	Histology	Drug	Response[b]	PFS	Site of biopsy	Treatment[c]	Response[d]	OS	EGFR mutation
1[a]	38	F	NS	IV	AD	Gef.	PR	7.1	Primary tumor	Docetaxel	SD	14.5	del E746-A750
2[a]	57	M	NS	IV	AD	Gef.	SD	5.1	Primary tumor	Docetaxel	PD	8.1	Wild type

[a] Patients with paired specimens; [b] Best response to EGFR TKIs; [c] Treatment following TKI resistance; [d] Response to treatment following TKI resistance

PFS progression free survival (months); *OS* overall survival (months); *M* male; *F* female; *NS* non-smoker; *AD* adenocarcinoma; *Gef.* gefitinib; *PR* partial response; *SD* stable disease; *PD* progressive disease

Table 6 Characteristics of TKI-naïve or-resistant patients with c-MET amplification

No.	Age	Sex	Smoking	Stage	Histology	Drug	Response[b]	PFS	Site of biopsy	Treatment[c]	Response[d]	OS	EGFR mutation
1	52	M	S	IB	LC	/	/	/	Primary tumor	/	/	49.8	Wild type
2	33	M	NS	IB	SQ	/	/	/	Primary tumor	/	/	36.7	Wild type
3	50	M	S	IV	AD	Gef.	SD	1.4	Lymph node	BSC	PD	2.3	Wild type
4	50	M	NS	IIIA	SQ	Erl.	SD	2.6	Primary tumor	RT	PD	7.7	Wild type
5[a]	66	M	S	IV	AD	Erl.	PR	8.2	Lymph node	Docetaxel	SD	13.9	Wild type

No.1 and 2 were TKI-naïve cases; No. 3~5 were TKI-resistant cases

[a] Patients with paired specimens; [b] Best response to EGFR TKIs; [c] Treatment following TKI resistance; [d] Response to treatment following TKI resistance

PFS progression free survival (months); *OS* overall survival (months); *M* male; *F* female; *S* smoker; *NS* non-smoker; *LC* large cell carcinoma; *SQ* squamous cell carcinoma; *AD* adenocarcinoma; *Gef.* gefitinib; *Erl.* erlotinib; *PR* partial response; *SD* stable disease; *PD* progressive disease; *BSC* best supportive care; *RT* radiotherapy

our data was not detected in the population of TKI-naïve patients, but 48.3% (14/29) of the enrolled TKI-resistant patients. In accordance with literature [21, 22], results of this study also suggested that the Scorpions ARMS method was more sensitive than direct sequencing for detecting EGFR T790M mutation.

Recent findings revealed that T790M mutation often occurred concomitantly with EGFR activating mutations [6, 19, 23]. Our data showed most of the T790M positive patients had EGFR activating mutations and were frequently associated with adenocarcinoma and non-smoker. Although a few subcloning analyses said T790M mutation was observed in the clones of wild-type EGFR [4, 20], few reports found T790M mutation occurred in EGFR wild-type NSCLC patients after development of TKI resistance. Of interest, we detected three patients with T790M mutation in the group of wild-type EGFR. The observation raised the possibility that T790M mutation was a secondary mutation in patients with wild-type EGFR under the selective pressure of TKI administration. The presence of T790M alone in TKI-resistant setting might be of clinical importance, as the second-generation irreversible TKIs, which were targeting acquired T790M mutation, might exert their effects on this subset of patients with wild-type EGFR after gefitinib or erlotinib treatment. And more studied are required to explore the mechanism of secondary T790M mutation in wild-type EGFR. Another possibility was that T790M mutation alone might exist in a small fraction of tumor cells before drug treatment, and the tumor cells harboring this mutation might be enriched over time during treatment. Previous study indicated that TKI-naïve cases harboring T790M mutation showed no response to gefitinib [24]. Conversely, two of three patients in our data responded to gefitinib, and the rest one achieved stable disease. Furthermore, T790M mutation occurred at a very low frequency in TKI-naïve patients [25, 26]. It was reasonable to speculate that T790M mutation in those three patients with wild-type EGFR may not exist before TKI treatment. The third explanation for the T790M mutation in TKI-resistant patients with wild-type EGFR might be the presence of a germ-line EGFR T790M mutation [27]. But the absence of T790M mutation in peripheral blood by our analysis minimized the possibility of germ-line T790M mutation.

As the occurrence of the EGFR T790M mutation can only explain part of the EGFR TKI-resistance mechanism, attention has been directed toward other resistance mechanisms. By constructing a gefitinib-resistant cell line (lung adenocarcinoma HCC827), Engelman et al. found that c-MET gene amplification was another major mechanism of TKI resistance in NSCLC [6]. In our analysis, c-MET amplification was detected in 17.2% of gefitinib- or erlotinib-resistant patients. This was in line with the data of Engelman and Bean [6, 23].

H820 cell, a lung adenocarcinoma cell line harboring EGFR T790M mutation and c-MET amplification implied the coexistence of these two genetic defects in TKI-resistant tumors. This was confirmed by a couple of *in vivo* studies [6, 23]. In our study, two patients with the coexistence of T790M mutation and c-MET amplification were non-smoker and showed adenocarcinoma. As both amplified c-MET and T790M mutated EGFR activated ErbB3 and PI3K/Akt in the presence of TKIs [6, 8], cooperative or dominant role of amplified c-MET remains unknown when it coexisted with T790M mutation in TKI resistance. Due to the small number of patients, more researches are warranted to investigate the significance of concurrent T790M mutation and c-MET amplification in TKI-resistant NSCLC.

It was reported that c-MET amplification were frequently detected concurrent EGFR activating mutation [6, 23]. Differently, we found c-MET amplification can occur with wild-type EGFR in TKI-resistant patients. And amplified c-MET showed no bias to smoking status or histology, which was consistent with the previous report [17]. As c-MET amplification also existed in the TKI-naïve population, it might be of interest to explore the possible differences between c-MET amplification in TKI-naïve patients and in TKI-resistant patients. We did the comparison between features of two early stage patients and three TKI-resistant patients. Clinicopathologic factors of c-MET amplification in TKI-naïve patients were indistinguishable from those in TKI-resistant patients. The similarity implied that c-MET amplification played a role in primary or acquired resistance to EGFR TKIs.

Previous evidence showed that time to progression was almost identical between patients with T790M mutation and those without T790M mutation [21]. However, there were no reports regarding the differential prognostic value of c-MET and the EGFR T790M mutation in TKI resistance. Our findings showed that the PFS and OS of patients with T790M mutation in the EGFR wild-type group seemed to be longer than c-MET amplified patients of wild-type EGFR. But the tendency requires careful interpretation due to the small sample size. The difference could be induced by sampling error. And it was also possible that time needed for accumulation of detectable T790M mutation was longer than that for activation of an alternative pathway (c-MET/ErbB3/PI3K/Akt) after treatment with EGFR TKIs. Thirdly, multiple underlying mechanisms may coexist with T790M or c-MET amplification and interfere with the comparison.

In conclusion, EGFR T790M mutation and c-MET amplification can occur in TKI-resistant NSCLC with wild-type EGFR, and these genetic defects might be related to different survival outcome. c-MET amplification in TKI-naïve or -resistant patients might share similarities in

clinicopathologic factors. In the management of EGFR TKI resistance, genetic analysis should be done if biopsy tumors are available, instead of only focusing on clinical features.

Acknowledgements This work was supported by the National Natural Science Foundation of China 30772531, the Foundation of Guangdong Science and Technology Department, 2007A032000002, and the Chinese Lung Cancer Research Foundation.

We thank Pasi A. Jänne (Dana-Farber Cancer Institute, Boston, MA, USA) for DNA samples of NSCLC cell lines HCC827 parental and HCC827 GR6. We thank Qiu-Xiong Lin and Dong-Lan Luo (Department of Pathology, Guangdong General Hospital) for their assistance in microdissection. We also thank the AstraZeneca Innovation Center China (ICC) in Shanghai for the excellent technical support.

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