EGFR and *K-RAS* mutations and *ERCC1*, *TUBB3*, *TYMS*, *RRM1* and *EGFR* mRNA expression in non-small cell lung cancer: Correlation with clinical response to gefitinib or chemotherapy

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Abstract. Personalizing medicines has refined the traditional treatments for non-small-cell lung cancer (NSCLC). In the present study, efforts towards personalizing delivery of care based on the status of *EGFR* and *K-RAS* mutations, and mRNA expression levels of *ERCC1*, *TUBB3*, *TYMS*, *RRM1* and *EGFR* by choosing appropriate treatments for 52 patients with NSCLC were discussed. Among these 52 NSCLC patients, there were 14 patients treated with gefitinib. Ten patients with *EGFR* exon 21 point mutations or exon 19 deletions had better treatment outcomes following gefitinib treatment (71.4%). There were 38 patients treated with platinum-based chemotherapy. Docetaxel-platinum based chemotherapy was chosen as the first-line treatment when the patients had low or median *ERCC1*/*TUBB3* expression and gemcitabine-platinum based chemotherapy was chosen when the patients had low or median *ERCC1*/*RRM1* expression. In total, 26 cases had mRNA expression levels of *ERCC1*/*TUBB3* or *ERCC1*/*RRM1* that could be used to predict the treatment outcomes of chemotherapy (68.4%). The present results indicated that the mutation status of *EGFR*, as well as the mRNA expression levels of *ERCC1*, *TUBB3* and *RRM1*, could be used as predictors of the response to gefitinib or chemotherapy.

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Introduction

Lung cancer, of which nearly 80-85% is diagnosed as non-small-cell lung cancer (NSCLC), is one of the leading causes of fatality worldwide (1). The adjuvant chemotherapy, including the combinations of platinum and cytotoxic agents (such as docetaxel and gemcitabine) and epidermal growth factor receptor (EGFR)-targeted therapy, such as gefitinib (AstraZeneca, London, UK), has become common treatments for NSCLC (2). However, drug resistance causes unsatisfactory clinical responses to these medicines. Therefore, it is essential to understand the molecular markers associated with resistance to these medicines, to aid oncologists in choosing the more effective anti-tumor drugs for patients to achieve the most advantageous potential outcomes.

The major molecular markers associated with clinical response to gefitinib or chemotherapy in NSCLC include the status of *EGFR*, *K-RAS* mutations and mRNA expression levels of excision repair cross-complementing 1 (*ERCC1)*, class III β-tubulin (*TUBB3)*, thymidylate synthase (*TYMS)*, ribonucleotide reductase subunit M1 (*RRM1)* and *EGFR* (3-14). EGFR is a member of the ErbB family of receptors. Mutations within the tyrosine kinase domain of EGFR account for increased sensitivity to the tyrosine kinase inhibitor (TKIs), such as gefitinib (3,4). However, an insertion mutation and the point mutation T790M in exon 20 of EGFR are associated with resistance to TKIs (5). Previous studies have demonstrated that overexpression of *EGFR* was associated with increased sensitivity to gefitinib (6,7). K-RAS encodes a small guanosine triphosphate (GTP)-binding protein involved in numerous cellular processes, including proliferation and apoptosis (8). The wild-type K-RAS protein has intrinsic GTPase activity that catalyzes the hydrolysis of bound GTP to GDP. Mutations within the *K-RAS* gene, 98% in codons 12, 13 or 61, result in locking of oncogenic K-RAS into the GTP-bound state and lead to the constitutive activation of RAS signaling. *K-RAS* mutations are associated with resistance to gefitinib (9). ERCC1 is a DNA repair endonuclease responsible for the 5'-incision during DNA excision repair. Overexpression of ERCC1 is correlated to resistance to platinum-based chemotherapy (10). TUBB3 encodes a class III member of the β tubulin protein family. Clinical studies have

shown that high TUBB3 expression is associated with resistance to docetaxel and paclitaxel (11,12). TYMS is the enzyme used for DNA synthesis and repair. The high TYMS expression is associated with resistance to fluorouracil in NSCLC (13). RRM1 is one of the subunits of ribonucleoside-diphosphate reductase, which is essential for the production of deoxyribonucleotides prior to DNA synthesis. The high RRM1 expression in NSCLC is connected with resistance to gemicitabin-based therapy (14).

In the present study, the mutation status of *EGFR* and *K-RAS* genes, as well as the mRNA expression levels of *ERCC1*, *TUBB3*, *TYMS*, *RRM1* and *EGFR* genes on the tumor tissue samples from 52 NSCLC patients were analyzed. The patients were treated with gefitinib or platinum-based chemotherapy according to the status of these molecular markers. The associations of the status of these molecular markers and corresponding clinical responses were evaluated to determine whether these biomarkers could be used as predictors of the response to gefitinib or chemotherapy.

Materials and methods

Patients and study design. Patients were recruited by the Beijing First Affiliated Hospital of PLA General Hospital (304 Hospital, Beijing, China) between January 2013 and June 2014. A total of 52 patients who underwent surgery for NSCLC were enrolled in the study. The patient clinical characteristics are listed in Table I. A total of 52 tissue samples were obtained following surgery. The tissue samples were fixed in 10% neutral formalin for 16 h, desiccated and embedded in paraffin. The diagnosis of NSCLC was based on the cytological or histological findings and histological types were determined according to the World Health Organization criteria (15). For each formalin-fixed paraffin-embedded (FFPE) tissue sample, the tumor tissues had been cut by microdissection techniques and sent to the Guangzhou SurExam Medical Test Center for *EGFR* and *K-RAS* mutations, and *ERCC1*, *TUBB3*, *TYMS*, *RRM1* and *EGFR* mRNA expression analysis.

The NSCLC patients were administered with the platinum-based chemotherapy or gefitinib, according to the status of *EGFR* and *K-RAS* mutations, and the mRNA expression levels of *ERCC1*, *TUBB3*, *TYMS*, *RRM1* and *EGFR*. Computed tomography (CT) scans were performed at 4 weeks after treatments. The clinical responses to treatments were classified as progressive disease (PD), stable disease (SD), complete response (CR) and partial response (PR) according to the Response Evaluation Criteria in Solid Tumors criteria (15). The correlation of clinical responses and status of the biomarkers were analyzed. The study was approved by the Ethics Committee of the 304 Hospital. Written informed consent was obtained from all the patients enrolled.

DNA extraction and mutation analysis of EGFR and K-RAS. The analysis of the *EGFR* and *K-RAS* mutation status was performed at SurExam Medical Test Center. The Maxwell® system (Promega Corp., Madison, WI, USA) was used to extract DNA from paraffin-embedded tissues. The status of *EGFR* mutations in exons 18, 19, 20 and 21 and *K-RAS* mutations in codon 12, 13 and 61 was screened by SurPlex®-xTAG70plex (SurExam Biotech Co Ltd. Guangzhou, Guangdong, China). The method includes five steps (16).

Table I. Patient clinical characteristics.

Variables	Patients
Age, median years (range)	59 (39-78)
Gender, n (%)	
Male	38 (73.1)
Female	14 (26.9)
Smoker, n (%)	
Yes	33 (63.4)
No	19 (36.6)
Histology, n (%)	
Adenocarcinoma	28 (53.8)
Non-adenocarcinoma	24 (46.2)
Differentiation, n (%)	
Well and moderate	31 (59.6)
Poor	21 (40.4)
Stage, n (%)	
II	15 (28.8)
III	28 (53.8)

ERCC1, TUBB3, TYMS, RRM1 and EGFR mRNA expression analysis. *ERCC1*, *TUBB3*, *TYMS*, *RRM1* and *EGFR* mRNA expression analysis was performed by the multiplex branched-DNA technology at SurExam Medical Test Center. This technology includes a sandwich nucleic acid hybridization method in which mRNAs are captured through cooperative hybridization of multiple probes and subsequently coupled with a fluorescence signal amplification system (17). The signals were detected by the Luminex 200 system, as described previously (17).

Statistical analysis. The data were analyzed using SPSS 19.0 software package (IBM Corp., Armonk, NY, USA). The correlation between gene mutation status and mRNA expression levels was evaluated by Spearman correlation coefficients. $P<0.05$ was considered to indicate a statistically significant difference.

Results

EGFR and K-RAS mutations and clinical responses to gefitinib. *EGFR* mutations were detected in 16 (31%) of the 52 FFPE samples (Table II). Ten samples had *EGFR* exon 21 L858R point mutations, 5 were exon 19 deletions and 1 had L858R and exon 20 T790M point mutations. *K-RAS* mutations were detected in 5 (10%) of 52 FFPE samples (Table II). Four samples had *K-RAS* codon 12 point mutations and the other sample was a codon 61 point mutation. Among these 52 NSCLC patients, there were 14 patients treated with gefitinib. The treatment outcomes indicated that there were 5 patients with PR, 8 with SD and 1 with PD. Four of the 5 patients with PR had exon 21 L858R point mutations and the other had exon 19 deletions. However, 1 patient with PD also had EGFR mutations. Among the 8 patients with SD, 5 had

Table II. Status of *EGFR* and *K-RAS* mutations, and mRNA expression levels of *ERCC1*, *TUBB3*, *TYMS*, *RRM1* and *EGFR* on the tumor tissue samples of 52 NSCLC patients and corresponding clinical responses.

Case no.	*EGFR* mutation status	*K-RAS* mutation status	mRNA expression levels					Treatments	Clinical responses	Whether biomarkers predict treatment outcomes
			ERCC1	*TUBB3*	*TYMS*	*RRM1*	*EGFR*			
1	L858R	wt	Median	High	High	Median	High	Gefitinib	SD	Y
2	L858R	wt	Low	Median	Low	High	High	Gefitinib	PR	Y
3	wt	wt	Median	Low	High	High	Median	Docetaxel-platinum	PD	N
4	E746_A750del	wt	High	Low	Low	Median	High	Gefitinib	PD	N
5	L858R	wt	Low	Median	High	Median	High	Gefitinib	SD	Y
6	wt	wt	Low	High	Median	Low	High	Gemcitabine-platinum	PD	N
7	wt	wt	Median	Median	High	High	Median	Docetaxel-platinum	SD	Y
8	wt	Q61K	Median	Low	Low	Low	Low	Docetaxel-platinum	SD	Y
9	wt	wt	Median	High	High	Median	Low	Gemcitabine-platinum	SD	Y
10	wt	wt	Low	Low	Low	High	Low	Docetaxel-platinum	SD	Y
11	L858R,T790M	wt	Median	Low	High	Low	Low	Docetaxel-platinum	SD	Y
12	wt	wt	Median	Low	Low	Low	Low	Gemcitabine-platinum	SD	Y
13	wt	wt	Median	Low	High	Low	Low	Docetaxel-platinum	PD	N
14	wt	wt	Low	Median	Median	Low	Low	Gemcitabine-platinum	PR	Y
15	wt	wt	Low	Low	High	High	Median	Docetaxel-platinum	PD	N
16	wt	wt	Median	High	High	Low	Median	Gemcitabine-platinum	SD	Y
17	E746_A750del	wt	Low	Median	Median	Low	Low	Docetaxel-platinum	SD	Y
18	wt	wt	High	High	High	High	Median	Docetaxel-platinum	SD	N
19	wt	wt	Low	Low	High	High	Median	Docetaxel-platinum	SD	Y
20	L858R	wt	Low	Median	Low	Median	High	Gefitinib	SD	Y
21	wt	G12A	Low	Median	High	Low	Low	Docetaxel-platinum	SD	Y
22	wt	wt	Low	Low	High	High	Low	Docetaxel-platinum	SD	Y
23	wt	wt	Low	Median	Low	Median	Median	Docetaxel-platinum	PR	Y
24	L747_P753>S	wt	Median	Median	Median	High	High	Gefitinib	SD	Y
25	L858R	wt	Median	Low	High	Median	High	Gefitinib	PR	Y
26	wt	wt	Low	Median	Low	High	Low	Docetaxel-platinum	PR	Y
27	wt	wt	High	Median	Median	High	Low	Gefitinib	SD	N
28	wt	wt	Median	Low	High	High	Low	Docetaxel-platinum	PD	N
29	L858R	wt	High	High	High	Median	High	Docetaxel-platinum	SD	N
30	wt	wt	Low	Median	High	Median	Low	Docetaxel-platinum	PR	Y
31	wt	wt	Median	Low	High	High	Low	Docetaxel-platinum	PR	Y

Table II. Continued.

Case no.	*EGFR* mutation status	*K-RAS* mutation status	mRNA expression levels					Treatments	Clinical responses	Whether biomarkers predict treatment outcomes
			ERCC1	*TUBB3*	*TYMS*	*RRM1*	*EGFR*			
32	E746_S752>V	wt	Low	Median	High	Median	High	Gefitinib	SD	Y
33	L858R	wt	Median	Low	Low	Median	Median	Docetaxel-platinum	SD	Y
34	wt	wt	Low	Low	Median	Low	Low	Gemcitabine-platinum	SD	Y
35	wt	wt	High	High	High	High	Median	Docetaxel-platinum	SD	N
36	wt	wt	Median	Low	Low	High	High	Docetaxel-platinum	SD	Y
37	wt	wt	High	High	Median	High	High	Docetaxel-platinum	SD	N
38	wt	G12D	High	High	High	High	Low	Docetaxel-platinum	PD	Y
39	wt	wt	High	High	Median	High	High	Gefitinib	SD	N
40	wt	wt	Low	Median	High	Median	High	Docetaxel-platinum	SD	Y
41	wt	G12C	Low	High	High	Median	Low	Gemcitabine-platinum	SD	Y
42	wt	wt	High	Median	Median	High	Low	Gefitinib	SD	N
43	wt	wt	Median	Low	Median	High	Median	Docetaxel-platinum	PD	N
44	wt	wt	Low	Median	Low	Median	Median	Docetaxel-platinum	SD	Y
45	wt	G12V	Low	Median	High	High	Low	Docetaxel-platinum	SD	Y
46	L858R	wt	Median	Low	Median	High	High	Gefitinib	PR	Y
47	wt	wt	Median	High	Median	Low	Low	Gemcitabine-platinum	PD	N
48	L858R	wt	High	Median	Low	Low	Low	Gefitinib	PR	Y
49	wt	wt	Low	Median	High	High	Low	Docetaxel-platinum	SD	Y
50	wt	wt	Median	Low	High	High	Median	Docetaxel-platinum	PD	N
51	L747_A750>P	wt	Low	High	High	Low	Low	Gefitinib	PR	Y
52	L858R	wt	Median	Low	Low	High	High	Docetaxel-platinum	SD	Y

EGFR, epidermal growth factor receptor; *ERCC1*, excision repair cross-complementing 1; *TUBB3*, class III β-tubulin; *TYMS*, thymidylate synthase; *RRM1*, ribonucleotide reductase subunit M1; NSCLC, non-small-cell lung cancer; SD, stable disease; PR, partial response; PD, progressive disease.

Table III. Summary of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *EGFR* mRNA expression levels from 52 NSCLC patients.

	No. of patients (%)				
mRNA expression levels	*ERCC1*	*TUBB3*	*TYMS*	*RRM1*	*EGFR*
High	10 (19)	13 (25)	27 (52)	25 (48)	16 (31)
Median	20 (38)	19 (37)	12 (23)	14 (27)	12 (23)
Low	22 (43)	20 (38)	13 (25)	13 (25)	24 (46)

ERCC1, excision repair cross-complementing 1; *RRM1*, ribonucleotide reductase subunit M1; *TUBB3*, class III β-tubulin; *TYMS*, thymidylate synthase; *EGFR*, epidermal growth factor receptor; NSCLC, non-small-cell lung cancer.

EGFR exon 21 point mutations or exon 19 deletions and 3 had no mutations. This result indicated that the *EGFR* mutation status was associated with the efficacy of gefitinib. Among the 14 patients treated with gefitinib, the *EGFR* mutation status to predict the treatment outcomes could be used in 10 (71.4%).

mRNA expression levels of ERCC1, RRM1, TUBB3, TYMS and EGFR and clinical responses to chemotherapy. The summary of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *EGFR* mRNA expression levels is shown in Table III. Among these 52 NSCLC patients, the majority of patients had low (43%) *ERCC1*, low *TUBB3* (38%), high *TYMS* (52%), high *RRM1* (48%) and low *EGFR* (46%) expression. When the patients had low or median *ERCC1* expression and low or median *TUBB3* expression, they were suggested for docetaxel-platinum based chemotherapy treatment. When the patients had low or median *ERCC1* expression and low or median *RRM1* expression, they were suggested for gemcitabine-platinum based chemotherapy treatment.

There were 30 patients treated with docetaxel-platinum and 8 treated with gemcitabine-platinum (Table II). The treatment outcomes indicated that in the docetaxel-platinum treatment group, there were 4 patients with PR, 19 with SD and 7 with PD (Table II). The 4 patients with PR had low or median *ERCC1* expression and low or median *TUBB3* expression. Fifteen of the 19 patients with SD had low or median *ERCC1* expression and low or median *TUBB3* expression, but 4 of these had high *ERCC1* and *TUBB3* expression. One of 7 patients with PD had high *ERCC1* and *TUBB3* expression, however, 6 of these had low or median *ERCC1* expression and low or median *TUBB3* expression. In the gemcitabine-platinum treatment group, there was 1 patient with PR, 5 with SD and 2 with PD (Table II). The patient with PR had low *ERCC1* and *RRM1* expression. The 5 patients with SD had low or median *ERCC1* expression and low or median *RRM1* expression; however, the 2 patients with PD also had low or median *ERCC1* expression and low or median *RRM1* expression. Among these 38 patients treated with docetaxel-platinum or gemcitabine-platinum based chemotherapy, the mRNA expression levels of *ERCC1* and *TUBB3*, or *ERCC1* and *RRM1*, of 26 cases could be used to predict the treatment outcomes of chemotherapy (68.4%) (Table II).

Correlation between EGFR and K-RAS mutations and ERCC1, RRM1, TUBB3, TYMS and EGFR mRNA expression. The mRNA expression levels of *ERCC1*, *RRM1*, *TUBB3*, *TYMS* and *EGFR*, and the mutation status of *EGFR* and *K-RAS* were compared. Correlations were observed between the status of *EGFR* mutations and the mRNA expression levels of *EGFR* (P=0.001, r=0.437). The patients that had *EGFR* exon 21 L858R point mutations or exon 19 deletions were more likely to have high EGFR expression. No correlation was identified between the other genes.

Discussion

Chemotherapy has been the traditional treatment for the management of NSCLC. However, the resistance to chemotherapy leads to unsatisfactory treatment outcomes and prognosis. The discovery of molecular markers associated with the clinical responses to chemotherapy and introduction of targeted therapy have refined this traditional treatment for NSCLC. This has led to the concept of personalized medicines. In the present study, efforts in personalizing delivery of care based on the status of *EGFR* and *K-RAS* mutations and mRNA expression levels of *ERCC1*, *TUBB3*, *TYMS*, *RRM1* and *EGFR* in choosing appropriate treatments for patients with NSCLC were discussed.

In the present study, gefitinib was chosen as the first-line treatment when the patients were carrying mutations within the tyrosine kinase domain of *EGFR*, such as mutations in *EGFR* exon 18, 19 and 21, and no mutations were identified in *K-RAS* codon 12, 13 or 61. Docetaxel-platinum or gemcitabine-platinum based chemotherapy was chosen as the first-line treatment when the patients had low or median *ERCC1*/*TUBB3* expression, or low or median *ERCC1*/*RRM1* expression, respectively. Although the mRNA expression level of TYMS was analyzed, no fluorouracil-based chemotherapy had been administered. The majority of the patients with low or median *TYMS* expression also had low or median *ERCC1* expression or *EGFR* exon 19 mutations, therefore, platinum-based chemotherapy or gefitinib was administered for treatment. Three patients with wild-type EGFR were treated with gefitinib, as these patients were not suitable for platinum-based chemotherapy and they chose to undergo the EGFR-targeted therapy.

Mutations in *EGFR* and *K-RAS* were detected in 16 (31%) and 5 (10%) of the 52 FFPE samples, respectively. One sample had the *EGFR* exon 21 L858R and exon 20 T790M point mutations and no samples were identified to have both *EGFR* and *K-RAS* mutations. Statistical analysis indicated that the patients that had *EGFR* exon 21 L858R point mutations or

exon 19 deletions were more likely to have high *EGFR* expression. *EGFR* exon 21 L858R point mutations, exon 19 deletions or high *EGFR* expression suggested that the patients were sensitive to the gefitinib treatment.

Among these 52 NSCLC patients, there were 14 patients treated with gefitinib and a statistically significant association was revealed between the *EGFR* mutation status and treatment outcomes of gefitinib in 10 cases (71.4%). These findings are consistent with previous studies (18-20). In the other 4 cases, 1 male patient with PD had *EGFR* exon 19 deletions. This patient was >70 years old and had a smoking history of >20 years. It is possible that the advanced age and long smoking history of the patient affected the treatment outcomes. Three patients with SD had no *EGFR* or *K-RAS* mutations. There were 38 patients treated with chemotherapy; 30 were treated with docetaxel-platinum and 8 with gemcitabine-platinum. The mRNA expression levels of *ERCC1* and *TUBB3*, or *ERCC1* and *RRM1* could be used in 26 cases to predict the treatment outcomes of chemotherapy (68.4%). The clinical response rate of personalized medicine is more efficient than that of the traditional treatments (68.4 vs. 20-40%) (21).

In conclusion, although the sample size in the study was small, the findings indicated that the mutation status of *EGFR*, as well as the mRNA expression levels of *ERCC1*, *TUBB3* and *RRM1*, could be used as predictors of response to gefitinib or chemotherapy.

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