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Peripheral Blood for Epidermal Growth Factor Receptor Mutation Detection in Non-Small Cell Lung Cancer Patients

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Abstract

OBJECTIVE: It is important to analyze and track Epidermal Growth Factor Receptor (*EGFR*) mutation status for predicting efficacy and monitoring resistance throughout EGFR-tyrosine kinase inhibitors (TKIs) treatment in non-small cell lung cancer (NSCLC) patients. The objective of this study was to determine the feasibility and predictive utility of *EGFR* mutation detection in peripheral blood. *METHODS:* Plasma, serum and tumor tissue samples from 164 NSCLC patients were assessed for *EGFR* mutations using Amplification Refractory Mutation System (ARMS). *RESULTS:* Compared with matched tumor tissue, the concordance rate of *EGFR* mutation status in plasma and serum was 73.6% and 66.3%, respectively. ARMS for *EGFR* mutation detection in blood showed low sensitivity (plasma, 48.2%; serum, 39.6%) but high specificity (plasma, 95.4%; serum, 95.5%). Treated with EGFR-TKIs, patients with *EGFR* mutations in blood had significantly higher objective response rate (ORR) and insignificantly longer progression-free survival (PFS) than those without mutations (ORR: plasma, 68.4% versus 38.9%, $P = 0.037$; serum, 75.0% versus 39.5%, $P = 0.017$; PFS: plasma, 7.9 months versus 6.1 months, $P = 0.953$; serum, 7.9 months versus 5.7 months, $P = 0.889$). In patients with mutant tumors, those without *EGFR* mutations in blood tended to have prolonged PFS than patients with mutations (19.7 months versus 11.0 months, $P = 0.102$). *CONCLUSIONS:* *EGFR* mutations detected in blood may be highly predictive of identical mutations in corresponding tumor, as well as showing correlations with tumor response and survival benefit from EGFR-TKIs. Therefore, blood for *EGFR* mutation detection may allow NSCLC patients with unavailable or insufficient tumor tissue the opportunity to benefit from personalized treatment. However, due to the high false negative rate in blood samples, analysis for *EGFR* mutations in tumor tissue remains the gold standard.

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Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1]. Non-small cell lung cancer (NSCLC) comprises approximately 85% of all lung cancer cases, of which more than 70% are initially diagnosed with unresectable advanced disease [2,3]. Systemic treatment, including molecular-targeted therapy, plays a central role in the clinical management of NSCLC.

Small-molecule tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, specifically target epidermal growth factor receptor

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(EGFR) and generate much optimism in the treatment of NSCLC. *EGFR* mutations have been demonstrated to be the strongest predictive biomarkers for the efficacy of EGFR-TKIs [4–8]. Patients with *EGFR* activating mutations, mainly in-frame deletions in exon 19 (19Del) and L858R substitutions in exon 21, have dramatic tumor responses and favorable survival benefit from EGFR-TKIs [9,10]. However, most responsive patients would eventually experience progressive disease (PD). The secondary T790M mutation in exon 20 accounts for approximately 50% of the mechanism of acquired resistance [11]. Hence, it is of great clinical importance to analyze and track *EGFR* mutation status for predicting efficacy and monitoring resistance throughout EGFR-TKIs treatment in NSCLC patients.

EGFR mutation analysis is recommended in National Comprehensive Cancer Network clinical guidelines for NSCLC. Nevertheless, a national survey shows that only 9.6% of NSCLC patients with stage IIIb or IV disease had *EGFR*-related testing performed in China [12]. Partially because tumor tissue, the optimal DNA source for *EGFR* mutation analysis, is always difficult to obtain. Most NSCLC patients presenting inoperable advanced disease cannot provide surgical samples, whereas biopsy samples may not be sufficient for both pathologic examination and mutation analysis. Besides, many patients refuse repeated biopsy at the time of disease progression. However, peripheral blood of cancer patients frequently contains circulating free DNA (cfDNA) derived from tumor cells, which has been used to detect tumor-specific alterations [13]. Moreover, blood sampling is minimally invasive, readily accessible, relatively repeatable. Thus, using blood for *EGFR* mutation identification and follow-up shows promise.

Amplification Refractory Mutation System (ARMS) has been extensively used in large clinical trials, and has been proved to be a stable, highly sensitive and specific method for *EGFR* mutation detection in tumor tissue. This method was shown to be able to detect mutations in samples containing as little as 1% mutated DNA [4,14–16]. In this study ARMS was used to detect *EGFR* mutations in plasma, serum and tumor tissue samples of NSCLC patients. The objective of this study was to determine the feasibility and predictive utility of *EGFR* mutation detection in blood.

Patients and Methods

Patients

To be eligible for this study, patients were required to have pathologically confirmed NSCLC and available plasma, serum or tumor tissue for *EGFR* mutation analysis. 164 patients were enrolled in this study from October 2011 to October 2012 at Shanghai Pulmonary Hospital. Patients' clinicopathologic characteristics, treatment regimens, tumor responses and survival outcomes were recorded. Smoking history was based on records at patients' first clinic visit and having smoked more than 100 cigarettes in a lifetime was used to define smokers. Performance status was evaluated using the Eastern Cooperative Oncology Group criteria. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumours guidelines. Written informed consent was obtained from all participants, and provision of plasma, serum and tumor tissue for *EGFR* mutation analysis was optional. This study was approved by the Institutional Ethics Committee of Shanghai Pulmonary Hospital.

Sample Collection

Plasma was collected from 141 patients and serum from 108 patients. Plasma/serum was separated from 4 mL peripheral blood by centrifugation at 1,000 rpm for 10 min at 4°C within 4 hours after collection and stored at -80°C until DNA extraction. Tumor tissue obtained from 142 patients via biopsy was put into RNAlater solution (Ambion, Austin, Texas, USA) and stored at -80°C until DNA extraction. All tumor tissue samples went through pathologic evaluation to confirm the diagnosis of NSCLC.

DNA Extraction

DNA was extracted from 1 ml plasma/serum or 2-20 mg tumor tissue. The DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) was used to extract DNA according to the manufacturer's instructions. The concentration and purity of DNA were determined by NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, USA). DNA extracted from tumor tissue was standardized to 1 ng/μL, whereas cfDNA extracted from plasma/serum was used for *EGFR* mutation analysis immediately without standardization.

EGFR Mutation Analysis

The Human *EGFR* Gene Mutations Fluorescence Polymerase Chain Reaction Diagnostic Kit (Amoy Diagnostics, Xiamen, China), which is based on ARMS technology, was used to detect the 19Del, L858R and T790M mutation according to the manufacturer's instructions. Briefly, all reactions were performed in 25 μL volumes including 4.7 μL of template DNA, 0.3 μL of Taq polymerase and 20 μL of reaction buffer mix. Real-time PCR was carried out using MX3000P real-time PCR machine (Stratagene, La Jolla, CA, USA) under following conditions: (1) initial denaturation at 95°C for 5 min, (2) 15 cycles of 95°C 25 s, 64°C 20 s and 72°C 20 s, (3) 31 cycles of 93°C 25 s, 60°C 35 s and 72°C 20 s with fluorescence FAM and HEX reading at 60°C of each cycle in phase 3. Data analysis was performed with MxPro v4.10 (Stratagene, La Jolla, CA, USA). Cycle threshold (Ct) represents the threshold at which the signal was detected above background fluorescence. ΔCt values were calculated as the difference between the mutation Ct and control Ct. Positive results were defined as follows: (1) Ct is lower than 26, (2) Ct is higher than 26 and ΔCt is lower than the cut-off ΔCt value (11 for 19Del and L858R, 7 for T790M).

Statistical Analysis

SPSS statistical software, version 17.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze the data. The comparison of *EGFR* mutation rate among different sample types and the correlation between *EGFR* mutation status and clinicopathologic characteristics as well as response to EGFR-TKIs were evaluated using Chi-square test or Fisher's exact test. Cohen's kappa statistic and McNemar's test were used to analyze the concordance of *EGFR* mutation status between matched samples. Progression-free survival (PFS) with EGFR-TKIs treatment according to *EGFR* mutation status was estimated by Kaplan-Meier method and compared using log-rank test. A two-sided P value less than 0.05 indicated statistical significance.

Results

Patient Characteristics

In total, 164 Chinese patients with NSCLC were enrolled in this study from October 2011 to October 2012 at Shanghai Pulmonary