Translational Oncology
www.transonc.com

Volume 7 Number 6 December 2014 pp. 795–799 **795**

Suitability of Surgical Tumor Tissues, Biopsy, or Cytology Samples for Epidermal Growth Factor Receptor Mutation Testing in Non–Small Cell Lung Carcinoma Based on Chinese Population[1,2]

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Abstract

BACKGROUND: Epidermal growth factor receptor (EGFR) mutation status is crucial in treatment selection for non–small cell lung cancer (NSCLC) patients; however, the detection materials' availability remains challenging in clinical practice. In this study, we collected surgical resection tissues, lymph node biopsy, and cytological samples for EGFR mutation testing and investigated the associations between gene mutation and clinical characteristics. *METHODS:* Two hundred and seventy-six NSCLC adenocarcinoma specimens were collected, and highly sensitive amplification refractory mutation system method was implemented for EGFR mutation detection, with clinicopathologic characteristics involved in the final analysis. *RESULTS:* In the total of 276 samples, 96% (265/276) of tumors obtained evaluable EGFR mutation status, the frequency of mutation was 55.8% (148/265) in all specimens, and three different type samples shared a comparable successful testing rate: 97.4% (38/39) in surgical tumor tissues, 100% (108/108) in lymph node biopsy samples, and 92.2% (119/129) in cytological samples. EGFR mutation was significantly associated with sex, smoking history, lymph node metastasis status (N stage), primary tumor size, testing tissues origin, and sample type ($P < .05$). Multivariate analysis reconfirmed that smoking history and primary tumor size shared significant correlation with EGFR mutation after adjustment. *CONCLUSIONS:* Both lymph node biopsy and cytological samples were suitable surrogates for EGFR mutation detection in NSCLC compared with tumor tissues, gene status should be detected widely considering the high EGFR mutation rate, and nonsmoking history together with smaller primary tumor size was an independent indicator of EGFR mutation status.

Translational Oncology (2014) 7, 795–799

Introduction

Lung cancer causes the majority of cancer-related deaths all over the world, of which non–small cell lung cancer (NSCLC) comprises nearly 80% to 85% cases [1]; moreover, approximately 75% of patients presented locally advanced or distant metastasis when diagnosed [2]. Fortunately, with novel biological agents emerging for targeted therapy in cancer treatment, better response and longer survival were observed in many clinical trials [3–5]. These small molecular tyrosine kinase inhibitors (TKIs), such as gefitinib and icotinib, both appeared to have great advantages when compared with chemotherapy for first-line treatment in epidermal growth factor receptor (EGFR) mutant NSCLC patients. Furthermore, detecting EGFR status before TKIs usage as first-line therapy has been widely accepted [6,7].

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[1] Competing interests: The authors declare that they have no competing interests.

[2] Authors' contributions: X.H.H. participated in study design, collected data, carried out data analysis, and drafted the manuscript. Z.S.Z. and D.W. carried out the mutation analysis. Y.C.S. and S.W. performed the statistical analysis and participated in manuscript draft. L.W., Y.T.L., S. Y., and X.S.H. participated in samples collection. Y.F. participated in data collection. Y.S. participated in study design. Y.K.S. conceived the study, design and coordination the study, and draft the manuscript. All authors read and approved the final manuscript.

Received 21 August 2014; Revised 16 October 2014; Accepted 23 October 2014

1936-5233/14
http://dx.doi.org/10.1016/j.tranon.2014.10.008

EGFR mutation testing in clinical practice has been improved tremendously during the past decade; however, samples' availabilities remain challenging. Generally, tumor tissues are optimal for detecting based on sufficient tumor cells and genome DNA. While nearly 70% of lung cancer patients were diagnosed using biopsy or cytology specimens because of the unavailability of surgical tumor tissues in unresectable and advanced diseases [8,9]. Actually, sufficient diagnostic materials acquisition remains a problem in all populations; therefore, the necessity of diagnosis with small biopsy materials and cytological samples appears more and more important in clinical application. Current data confirm the cytology testing for EGFR mutation, with a promising concordance rate between tissues and cytological samples [10–13], which indicates that small specimens would play as appropriate surrogates in EGFR detection. As gene mutation testing methods increase dramatically, such as the second- or third-generation deep sequencing, Sanger sequencing has been replaced to some extent because of its limitations that include low sensitivity and longer time consumed [14,15], although it is still recognized as the "gold standard" in gene detection. Amplification refractory mutation system (ARMS) is a popular targeted real-time polymerase chain reaction (PCR)–based method for gene mutation detection, with a higher sensitivity, more convenient manipulation, and less turnaround time, which would be proper for EGFR testing especially in insufficient samples like lymph node biopsy and cytology specimens.

Nowadays, tumor tissues, biopsy, and cytological samples are the most common diagnostic materials for clinical testing, and EGFR mutation status appears to have a pivotal role in selecting patients who are most likely to derive benefits from TKI therapy. However, the discordance between EGFR mutation tests for several factors such as variation in tumor cell content and sample size differences remains a challenge [16,17]. In this study, we collected 276 NSCLC adenocarcinoma samples, and the EGFR mutation status was detected by ARMS. We aimed to investigate the EGFR mutation prevalence in different sample types; moreover, associations between gene mutations and clinicopathologic characteristics together with different testing results among these three samples types were analyzed.

Methods

Patients and Samples

From December 2012 to November 2013, a total of 276 NSCLC adenocarcinoma patients were enrolled in this study with available testing materials. Eligible patients had pathologically confirmed NSCLC adenocarcinoma according to American Joint Committee on Cancer seventh edition criteria and available tumor samples for gene mutation detection. All patients should be over 18 years old, and written informed consent was obtained from each patient before the study. The procedure was approved and supervised by the Institutional Review Board (IRB) of the Cancer Institute/Hospital of Chinese Academy of Medical Sciences and Peking Union Medical College. Smoking is defined as at least one cigarette per day or occasionally for at least 1 year, regardless of past or current status, and patients who had never smoked cigarettes during their lifetime were recognized as never-smokers.

DNA Extraction and Mutation Analysis

Hematoxylin and eosin staining and histologic analysis were used to identify the representative malignant cells in each specimen by two independent pathologists before experiments. Tissue or cell blocks were cut into 5-μm sections for formalin-fixed, paraffin-embedded samples, and DNA extraction was performed using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA was dissolved in 50 μl of deionized water and stored at −80°C until use. DNA concentration was measured with a NanoDrop2000 spectrophotometer (Thermo Scientific, Wilmington, USA) by detecting optical absorbance at 260 nm. Then the EGFR RGQ PCR Kit (Qiagen) was used for EGFR mutation detection with the ARMS/Scorpion assay, which allows testing of 29 known mutations for EGFR. PCR results were collected and analyzed according to the manufacturer's protocols.

Statistical Analysis

Statistical analysis was carried out by the SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL). Associations between clinicopathologic characteristics and EGFR status in all samples types were evaluated by the chi-square or Fisher exact tests, and only variables with statistical significance were subjected to final logistic regression analysis, using a backward stepwise (likelihood ratio) method with odds ratio (OR) calculated. The two-side significant level was set at $P < .05$ through the whole analysis process.

Results

Patients

We have enrolled 276 patients (128 male and 148 female with a mean age of 56 years) in this study, of which 39 (14.1%) samples were surgical resection tumor tissues, 108 (39.1%) were derived from lymph node biopsy, and 129 (46.8%) cases were cytological samples (98 needle aspiration biopsy, 29 transbronchial endoscopic biopsy, and 2 pleural effusion samples). The patients' demographic and clinicopathologic data are presented in Table 1.

EGFR Mutation Assessment

The EGFR mutation was detected successfully in 265 (96%, 265/276) samples; 148 (55.8%, 148/265) harbored an EGFR mutation, of which 68 (25.7%, 68/265) were deletion in exon 19 and 70 (26.4%, 70/265) were L858R in exon 21. T790M mutation was found in three samples; moreover, two of these coexisted with an L858R mutation. Other mutant types included G719X in exon 18, L861Q in exon 21, or combined mutation deletion (exon 19) with L858R. The spectrum of these mutations was shown in Table 2. All lymph node biopsy samples were detected successfully (108/108, 100%), whereas the test success rates of tumor tissue samples (38/39, 97.4%) and cytological samples (119/129, 92.2%) were somewhat lower. These failed 11 samples included 1 surgical resection tumor tissue and 10 cytological specimens that contained 8 transthoracic needle biopsy samples and 2 transbronchial endoscopic biopsy samples. Before we performed the EGFR mutation detection, strict quality control included DNA concentration, and A260/A280 absorbance ratio was calculated. Nearly all of these samples' DNA concentration was lower than 6.0 ng/μl (only one sample's DNA concentration was 10.0 ng/μl), and most of the absorbance ratio appeared aberrant (seven samples with an A260/A280 absorbance ratio over 2.4 and one sample was lower than 1.7). These 11 samples did not pass the Qiagen kit positive control; therefore, mutation detection was not performed. The mutant rates for detected samples were 63.2% (24/38) in tumor tissues, 46.3% (50/108) in lymph node biopsy samples, and 62.2% (74/119) in cytological samples, respectively. And the mutation rates were significantly different in these three type samples ($P = .034$,Table 1).