Original Article

Low Correspondence of EGFR Mutations in Tumor Tissue And Paired Serum of Non-Small-Cell Lung Cancer Patients

Guo-hong Song, Jun Ren*, Li-jian Zhang, Li-jun Di, Yan-hua Yuan, Jing Yu, Jun Jia

Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education); Department of Medical Oncology, Peking University School of Oncology, Beijing Cancer Hospital & Institute, Beijing 100142, China

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ABSTRACT

Objective: Epidermal growth factor receptor (EGFR) mutations are strong determinants of tumor response to EGFR tyrosine kinase inhibitors in non-small-cell lung cancer (NSCLC) patients. The aim of this study was to evaluate the correspondence between EGFR mutations in non-small-cell lung cancer tissues and in circulating DNA.

Methods: The research was conducted in 50 non-small-cell lung cancer patients who had undergone curative surgery, and in whom both serum and neoplastic tissues were available. Meanwhile sera of 33 cases of advanced NSCLC patients were also analyzed. DNA were extracted from each sample. Mutations of EGFR in exon18-21 were examined by PCR amplification method and direct sequencing.

Results: EGFR mutations were detected in 15 (30%) of 50 neoplastic tissue samples, 6 cases were in-frame deletion del E746-A750 in exon19, 9 cases were substitution in exon 21 (all were L858R except one was L861Q), but no mutated DNA resulted in paired serum circulating DNA samples of 50 resectable patients. As the 33 advanced NSCLC patients, EGFR mutations were detected in only 2 serum circulating DNA samples, all were L858R mutation in exon 21.

Conclusion: These data indicated that it was difficult to identify EGFR mutations in circulating DNA of NSCLC patients. The use of EGFR mutation in serum as a clinical method for decision making of TKI therapy is unsatisfactory.

Key words: Circulating DNA; Epidermal growth factor receptor (EGFR); Mutation; Non-small-cell lung cancer (NSCLC)

INTRODUCTION

Lung cancer is a major cause of cancer-related mortality worldwide and is expected to remain a major health problem in the future[1]. Targeting the epidermal growth factor receptor (EGFR) is an encouraging strategy for the treatment of non-small-cell lung cancer (NSCLC) as EGFR has been found to be expressed, sometimes strongly, in NSCLC tumors[2]. Studies have reported that EGFR mutations are strong determinants of tumor response to EGFR tyrosine kinase inhibitors (TKI) in NSCLC patients[3–6]. Most EGFR mutations have been identified retrospectively from operative resected tumor samples. However, it is sometimes difficult to obtain tumor samples from patients with inoperable NSCLC in prospective studies; thus, it is necessary to establish a method to detect mutant EGFR from other more readily accessible patient samples.

The finding that tumors are capable of shedding nucleic acids into the bloodstream has opened new areas in cancer research[7, 8]. Large amounts of tumor-derived DNA may be released from a tumor mass in which cell necrosis or lysis of tumor cells occurs, resulting in a very elevated serum DNA

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*Corresponding author.
E-mail: renjun@bjcancer.org

concentration[9–12]. Although considerably degraded, DNA can be recovered from a patient's serum or plasma and used as a surrogate source of tumor DNA. Accordingly, the number of studies evaluating the potential use of serum or plasma DNA in cancer diagnosis and prognosis has increased steadily in the past decade. In the case of non-small-cell lung cancer (NSCLC), some reports showed that K-ras point mutations and P53 mutation can be found in circulating DNA and were identical to the mutations found in the tumors[13, 14], but the results were not the same[15]. As to EGFR mutation in circulating DNA, there is only two reports showing the EGFR mutation in the serum consistent with those in the tumor samples of advanced NSCLC patients[16, 17]. The detection of EGFR mutations in serum DNA may provide a noninvasive and repeatable source of genotypic information that might facilitate clinical decision making at the time of diagnosis and in the later course of the disease, especially in patients with NSCLC treated with TKIs.

The purpose of the present study was to evaluate the correspondence of EGFR mutation of NSCLC patients in whom it was possible to analyze both tissue samples and serum.

MATERIALS AND METHODS

Patients

Clinical samples comprised 50 tumor samples that had been resected from non-small cell lung cancer patients (27 men and 23 women) at the time of operation From March 2004 to July 2006 and immediately frozen at -80°C. Peripheral Blood samples were obtained from each patient before surgery. Meanwhile blood samples of 33 cases of advanced NSCLC patients were also available. Separated serum was stored at -80°C until use. Written informed consent was obtained before collecting samples from each individual. Histopathologic diagnosis for these 50 resectable tumors included adenocarcinoma (n=24), squamous cell carcinoma (n=20), bronchioloalveolar carcinoma (n=2), large-cell carcinoma (n=2), and adenosquamous carcinoma (n=2). No patients received preoperative antitumor therapy except two that had received two cycles of neoadjuvant chemotherapy. Staging for these patients resulted as follows: stage I (n=16), stage II (n=18) and stage IIIa (n=16), according to TNM staging system. Histopathologic diagnosis for the 33 advanced patients included adenocarcinoma (n=19), squamous cell carcinoma (n=11), bronchiolo-alveolar carcinoma (n=2), and adenosquamous carcinoma (n=1). We analyzed EGFR mutations in the tissue samples and investigated EGFR mutations in the serum of all the patients.

DNA Extraction

DNA was extracted from tumor samples using a DNAeasy kit (U-gene biotechnology CO., LTD, China) according to the instructions of the manufacturer. The concentration and purity of the extracted DNA were determined by Beckman Coulter DU800 spectrophotometer.

DNA from serum samples of these patients was extracted using QIAamp (Qiagen, Basel, Switzerland) DNA blood kit. The extraction was performed according to the supplier's recommendations. The resulting DNA was eluted in 50 μl buffer (provided by the kit). The serum DNA can not be quantified by spectrophotometer because of the very low amount of DNA.

PCR Amplification and Sequence Analysis

Amplification of exons 18 to 21 were done in duplicate for each sample obtained from serum and tissue specimens. Primer sequences for EGFR exons 18 to 21 were obtained from Shigematsu H, et al.[6]. All PCR assays were carried out in a 50 μl volume that contained 200 ng of tissue genomic DNA or 6 μl of serum DNA and 2.5 units of Taq DNA polymerase (Takara). DNA was amplified for 35 cycles at 95°C for 45 s, 65°C for 30 s, and 72°C for 45 s, followed by a 10 min extension at 72°C. PCR products were electrophoresed in 1% agarose gel .Only those PCR products producing a positive band were send to Beijing AuGCT Biotechnology Co., Ltd, China for purification and sequence analysis. Sequencing was carried out in an automated DNA analyzer (ABI Prism 3730; PE Biosystems). The sequences were compared with the GenBank-archived human sequence for EGFR (accession no.AY588246). All sequence variants were confirmed by sequencing the products of independent PCR amplifications.

RESULTS

PCR Analysis

Exon 18 to 21 could be amplified in all tissue samples, 42 of 50 resectable patients serum and 24 of 33 advanced patients serum. Each PCR product