Effect of icotinib on advanced lung adenocarcinoma patients with sensitive EGFR mutation detected in ctDNA by ddPCR

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Background: Whether or not EGFR mutation status detected by ddPCR in plasma predicts the effect of icotinib on patients with advanced lung adenocarcinoma was determined.

Methods: Plasma and matched tissue specimens from patients with advanced lung adenocarcinoma were collected prior to icotinib treatment. The ARMS method was used to detect EGFR mutation status in DNA extracted from tissue specimens, while the EGFR mutation status in ctDNA extracted from plasma specimens was determined by ddPCR. The therapeutic effects of icotinib were compared between patients with EGFR-activating mutations detected by ddPCR in ctDNA and ARMS in tissue DNA.

Results: EGFR mutation status was detected in 96 tissue and 100 plasma specimens. The sensitivity and positive predictive value of 19del detected in ctDNA by ddPCR was 70.97% (22/31) and 44.90% (22/49), respectively. The positive predictive value was 84.62% (22/26) and the sensitivity was 53.66% (22/41) for the L858R mutation. For the common sensitive EGFR mutations, ddPCR had a positive predictive value of 77.19% (44/57) and a sensitivity of 48.89% (44/90). Patients with sensitive EGFR mutations in ctDNA had objective response and disease control rates (DCR) similar to patients who had sensitive EGFR mutations in tissues detected by ARMS when treated with icotinib (57.14% vs. 51.51% and 92.86% vs. 90.91%, respectively).

Conclusions: Patients with sensitive EGFR mutations in plasma specimens detected with ddPCR had a higher ORR and DCR compared with patients with sensitive EGFR mutations in tissue detected with the ARMS method.

Keywords: NSCLC; EGFR-tyrosine kinase inhibitors (EGFR-TKIs); T790M; ddPCR

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide and morbidity is expected to increase in the coming decades (1). Adenocarcinoma is the most common histologic type of lung cancer (2). Radical surgery is still the most effective method for treating early lung adenocarcinoma, but post-operative relapse occurs with or without tumor metastasis, and most patients with lung adenocarcinoma are diagnosed with advanced-stage disease. The response rate of platinum-based chemotherapy for advanced or recurrent patients is only 20–35%, and the median survival time is only 8–11 months (3,4). In recent years, it has been confirmed that EGFR-tyrosine kinase inhibitors (TKIs) prolong the median progression-free survival time to >8 months in lung adenocarcinoma patients with activating EGFR mutations, and the response rate has increased to 56–74% (5-9). Indeed, EGFR-TKIs are the first-line standard treatment for lung adenocarcinoma patients with activating EGFR mutations.

The relationship between EGFR mutation status and the therapeutic efficacy of EGFR-TKIs has been verified. Assessing the presence of EGFR mutations is a critical step in therapeutic decision-making for lung adenocarcinoma patients (10). Tumor tissue is still the recommended source for detecting EGFR, but because quality tissue samples are not always available it is not possible to conduct EGFR testing for every patient in need (11,12). Thus, alternative, easily acquired specimens from patients and feasible methods of detection are essential. Recently, circulating tumor DNA (ctDNA) has been shown to be a suitable alternative sample because ctDNA can be obtained non-invasively (13). Several technologies have been developed for EGFR detection using ctDNA (14-19). Of these technologies, the droplet digital polymerase chain reaction (ddPCR) is a method with the following characteristics: high sensitivity; quantitative analysis; and easy detection of gene mutations.

Using EGFR mutation-positive cell DNA, ddPCR has expanded to 0.02% sensitivity (20). EGFR detection by ddPCR in plasma ctDNA could achieve a sensitivity of 70–81%, specificity of 85–100%, and concordance of 86–94% compared to tumor EGFR status (21-23); however more detailed clinical validation is needed to evaluate plasma specimens using ddPCR for EGFR detection, especially with respect to the diagnostic and prognostic significance compared to tissue specimens. In this study we compared the EGFR detection results and analyzed the clinical outcomes following icotinib treatment, as determined by ARMS for tissue specimens and ddPCR for matched plasma specimens. The purpose of the current study was to provide clinical evidence to verify the value of predicting EGFR mutation status detected by ddPCR in plasma.

Methods

Patients and treatment

In this study, patients who diagnosed with stage IV lung adenocarcinoma and sensitive EGFR mutations based on tissue specimens or ctDNA were enrolled from January 2016 to December 2017 at Zhejiang Rongjun Hospital were enrolled in the current study. The study was reviewed and approved by the Institutional Ethics Committee of this hospital. Informed consent was obtained from each patient prior to testing. Demographic, epidemiologic, and pathologic information were obtained from patient medical records. None of the patients received targeted therapy, radical surgery, or radiation treatment within 28 days prior to sample collection. The enrolled patients were given 125 mg of icotinib three times per day on the basis of the EGFR mutation detection results and patient preference. Chest CT scans were performed 1 month after beginning EGFR-TKI treatment. The response to EGFR-TKIs was evaluated according to the Response Evaluation Criteria in the Solid Tumor guideline (version 1.1).

DNA extraction

Plasma was collected from every patient before therapy. Ten milliliters of whole venous blood were collected into tubes with an anti-coagulant which also contained a ctDNA protective agent (Righton, Shanghai, China), stored at 4–25 °C, and centrifuged (1,800 g for 10 min at 4 °C) as soon as possible. A Qiamp® Circulating Nucleic Acid Kit (Qiagen, Duesseldorf, Germany) was used to extract ctDNA from plasma specimens. Tissue DNA was extracted using an AmoyDx® Tissue DNA Kit (Amoydx, Xiamen, China). DNA quality was verified using a nucleic acid quantitative instrument (Thermos City, MA, USA).

Detection of EGFR mutations in tissue DNA and ct DNA

EGFR mutation status in tissue DNA was detected using the ARMS method. The tests were carried out according to the manufacturer’s protocol using the AmoyDx EGFR
29 Mutation Kit (Amoydx) with the MX3000P real-time PCR system (Stratagene, La Jolla, CA, USA). The results were identified according to the criteria defined by the manufacturer’s instructions. EGFR mutation status in ctDNA was detected using ddPCR. Scientists affiliated with the Righton Testing Institute, who conducted the ddPCR, were blinded to the tissue results.

**Statistical and database analyses**

The objective response rate (ORR) and disease control rate (DCR) after TKI treatment were analyzed by chi-square or Fisher’s exact tests. The identified mutation status (M+ or M−) in both samples was the basis of comparison of ctDNA versus tumor EGFR mutations before treatment. The data were analyzed using GraphPad Prism (version 7.0; GraphPad, city, CA, USA), and statistical significance was indicated by a P value <0.05.

**Results**

**Clinical and pathological characteristics of patients**

Sensitive EGFR mutations were detected in 100 patients from four hospitals. All 100 patients provided sufficient plasma specimens for EGFR detection, but tissue specimens were not available or insufficient for EGFR detection in four patients prior to treatment. The patient age range was 26–83 years and the median age was 59 years. The clinical and pathologic profiles of the enrolled patients are listed in Table 1.

**EGFR mutation status**

Sensitive EGFR mutations were detected in tissue specimens from 92 patients prior to treatment. Of the 92 patients, common sensitive mutations were detected in 87 patients; 49 patients had 19del mutations, 41 patients had L858R mutations, and three patients had both 19del and L858R mutations. Five patients had rare sensitive mutations; three patients had G719X mutations and two patients had L861Q mutations (Table 2). T790M mutations existed in two patients who had L858R mutations. An EML4-ALK rearrangement was detected in one patient with a 19del mutation. EGFR mutations were detected in plasma ctDNA from 100 patients by ddPCR prior to treatment. Fifty-eight patients had common activating EGFR mutations. Of the 58 patients, 33 had 19del mutations, 28 had L858R mutations, both 19del and L858R mutations existed in three patients, one patient had both T790M and L858R mutations, and two patients were shown to have no other mutations. No patients had rare sensitive mutations (Table 2).

**Comparison of EGFR mutation status in plasma and tumor tissue**

EGFR mutation status was determined in the plasma and tissue specimens from 96 patients. For four patients, the EGFR status was determined in their plasma specimens, but not in their tissue specimens from four patients. The EGFR status was not detected in tissue specimens, but not plasma specimens from any patients.

Thirty-three plasma specimens were positive for 19del mutations. The matched tissue results were lost in two of the 33 cases, nine had false-positive results (nine patients had positive plasma specimen results, but negative tissue specimen results); the positive predictive value for 19del mutations was 70.97% (22/31) and the sensitivity was
Of 28 plasma specimens with L858R mutations, there were no matched tissue results in two cases and four cases had L858R mutations in plasma specimens, but not in tissue specimens. The positive predictive value for L858R mutations detected in plasma by ddPCR was 84.62% (22/26) and sensitivity of 53.66% (22/41). For the common sensitive EGFR mutations, the positive predictive value was 77.19% (44/57) and the sensitivity was 48.89% (44/90; Table 3).

Table 3 Comparison of EGFR mutation status in plasma specimens and matched tissue specimens

| EGFR mutation status, plasma, n | EGFR mutation status, tissue, n |
|---------------------------------|---------------------------------|
| Positive                        | Positive                        |
| 19del                           | 22                              |
| Negative                        | 27                              |
| Total                           | 49                              |
| L858R                           | Positive                        |
| 22                              |
| Negative                        | 19                              |
| Total                           | 41                              |

NA, not available.

Figure 1 Comparison of ORR and DCR in the plasma mutation group and tissue mutation group. ORR, objective response rate; DCR, disease control rate.

In 13 patients, including nine with 19del mutations and four with L858R mutations, EGFR mutations were detected in plasma samples, but not in the paired tumor tissues. This finding may reflect tumor heterogeneity or biopsy bias (26,27). In another 46 patients (27 with 19del mutations and 19 with L858R mutations), EGFR mutations were present in tissue specimens. Thirty-four patients had partial responses (PR) to icotinib treatment; of these patients, 16 had 19del mutations, 15 patients had L858R mutations, and the other three had both mutations. The total ORR was 51.52% (34/66). The total DCR was 90.91% (60/66). With the exception of patients who had a PR, 26 had disease stabilization (SD); specifically, 14 patients had 19del mutations, nine had L858R mutations, two had G719X mutations, and one patient had a L861Q mutation. Forty-two of 66 patients had sensitive EGFR mutations detected in plasma specimens. The total ORR and DCR was 57.14% (24/42) and 92.86% (39/42), respectively. Twenty-four of the 42 patients had a PR, including 12 patients with 19del mutations, 11 patients with L858R mutations, and one patient with both mutations. Nine patients with 19del mutations and six patients with L858R mutations had SD (Figure 1).

Discussion

EGFR status is a significant prognostic factor for NSCLC patients who receive EGFR-TKI treatment. Plasma has been shown to be effective as an addition to tissue specimens for EGFR testing (24,25). In this study, EGFR status in plasma ctDNA detected by ddPCR was compared to matched tissues detected by the ARMS method, and the efficacy of icotinib on patients with sensitive EGFR mutations detected in plasma ctDNA was explored.

In this study, 100 plasma specimens and 98 matched tissue specimens were collected for EGFR detection by ddPCR and ARMS, respectively. The sensitivity and positive predictive value of 19del mutations detected in plasma by ddPCR were 44.90% (22/49) and 70.97% (22/31), respectively. We detected L858R mutations in plasma by ddPCR with a positive predictive value of 84.62% (22/26) and sensitivity of 53.66% (22/41). For the common sensitive EGFR mutations, ddPCR had a positive predictive value of 77.19% (44/57) and a sensitivity of 48.89% (44/90). The ddPCR method failed to detect rare sensitive mutations. Negative results existed in the five plasma specimens with rare sensitive EGFR mutations detected in matched tissue specimens.

Relationship between EGFR mutation status in plasma and tissue specimens with EGFR-TKI efficacy

Of the 100 patients in whom plasma or tissue specimens were EGFR sensitive mutation-positive, 66 received icotinib treatment. All 66 patients had sensitive EGFR mutations
in tissue specimens, but not in plasma specimens. Tumors with \( \text{EGFR} \) mutations may not or rarely release mutant tumor DNA fragments into the blood; thus, no mutations cannot be detected in plasma (28).

The goal of \( \text{EGFR} \) mutation testing in the clinic is to select patients who might benefit from \( \text{EGFR-TKIs} \). Therefore, we examined the efficacy of icotinib on patients who had sensitive \( \text{EGFR} \) mutations in plasma based on ddPCR. Compared with patients who had sensitive \( \text{EGFR} \) mutations in tissue based on the ARMS method, the patients who had sensitive \( \text{EGFR} \) mutations in plasma specimens had a higher ORR and DCR (57.14\% vs. 51.51\% and 92.86\% vs. 90.91\%, respectively), but the difference was not statistically significant.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.10.48). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was reviewed and approved by the Institutional Ethics Committee of this hospital. Informed consent was obtained from each patient prior to testing.

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