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Personalized biomarkers to monitor disease progression in advanced non-small-cell lung cancer patients treated with icotinib

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ABSTRACT

Background: Disease-specific humoral immune response-related protein complexes in blood are associated with disease progression.
Methods: Thirty-one patients with stage IIIB and IV non-small-cell lung cancer (NSCLC) were administered with oral dose of icotinib hydrochloride (150 mg twice daily or 125 mg 3 times daily) for a 28-continuous-day cycle until diseases progressed or unacceptable toxicity occurred. The levels of immunoinflammation-related protein complexes (IIRPCs) in a series of plasma samples from 31 NSCLC patients treated with icotinib hydrochloride were determined by an optimized native polyacrylamide gel electrophoresis.
Results: Three characteristic patterns of the IIRPCs, named as patterns a, b, and c, respectively, were detected in plasma samples from 31 patients. Prior to the treatment, there were 18 patients in pattern a consisting of 5 IIRPCs, 9 in pattern b consisting of six IIRPCs, and 4 in pattern c without the IIRPCs. The levels of the IIRPCs in 27 patients were quantified. Our results indicate that the time length of humoral immune and inflammation response (TLHIIR) was closely associated with disease progression, and the median TLHIIR was 22.0 weeks, 95% confidence interval: 16.2 to 33.0 weeks, with a lead time of median 11 weeks relative to clinical imaging evidence confirmed by computed tomography or magnetic resonance imaging (the median progression-free survival, 34.0 weeks, 95% confidence interval: 27.9 to 49.0 weeks).
Conclusions: The complex relationships between humoral immune response, acquired resistance, and disease progression existed. Personalized IIRPCs could be indicators to monitor the disease progression.

1. Introduction

Lung cancer is a leading cause of cancer-related mortality worldwide [1], which is mainly classified into non-small-cell lung cancer (NSCLC, about 85% of lung cancer) and small cell lung cancer (about 15%) [2]. Treatment plans have been prescribed to patients based on clinicopathological criteria. In recent years, advances in personalized lung cancer treatment have been made to targeting epidermal growth factor receptor (EGFR) with tyrosine kinase inhibitors (TKIs) (*e.g.*, erlotinib, gefitinib, and icotinib) in NSCLC [3]. Unfortunately, only a small number of the patients with advanced lung cancer responded to EGFR-TKI therapy, and finally also acquired drug-resistance [4]. Acquired drug-resistance to EGFR-TKI therapy was also associated with T790M mutation in exon 20 of EGFR and overexpression of hepatocyte growth factor [5–7]. Previous study on disease progression has revealed that inflammation regulated by immunoglobulin and immune complexes might be a functionally significant factor of cancer promotion and progression [8]. Previous study has also indicated that the development of EGFR-TKI-induced skin inflammation was associated with prolonged survival [9]. However, the mechanisms of these processes in the cancer development have not yet been fully understood. Recently, genomic alterations in solid cancers have been characterized by sequencing cell-free DNA in the blood of cancer patients [10], but it is impossible to use these assays to monitor each individual's disease progression due to their high cost. Clinical challenges in the treatment outcome of the patients with advanced lung cancer may require the determination of serological treatment response-related biomarkers to monitor disease progression and/or acquired drug-resistance and to improve therapeutic outcome. Serological biomarkers may be the products of cancer cells, tumor microenvironment, and host response, along with their dynamic interactions [11]. Therefore, it is important to develop less invasive methods to monitor the disease progression, instead of detecting tumor cells or tissue DNA. It has been observed that, in the advanced NSCLC patients

Abbreviations: NSCLC, non-small-cell lung cancer; IIRPCs, immunoinflammation-related protein complexes; TLHIIR, time length of humoral immune and inflammation response; TLDP, time length of the disease progression; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

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treated with gefitinib, serum surfactant protein D may be a new surrogate marker for predicting the response to gefitinib [12]. In addition, biomarkers should be of personal, less invasive, sensitive, and economic features, along with higher reproducibility. Our previous study has provided insight into the association of the levels and patterns of circulating immunoinflammation-related protein complexes (IIRPCs), which include immune-related proteins, inflammation-related proteins, and complement-related proteins [13], with cancers.

2. Materials and methods

2.1. Patients

From August 2007 to March 2010, 31 patients with advanced NSCLC were enrolled in stage I and II clinical trials of icotinib hydrochloride on the basis of the eligibility criteria as previously described (BPI-2009H) [14,15]. Briefly, eligible patients were required to have histologically or cytologically confirmed stage IIIB or IV NSCLC and had received chemotherapy based on the American Joint Committee on Cancer system, 6th edition at the tumor-node-metastasis classification of staging system. Measurable tumors with the response evaluation criteria in solid tumors (RECIST, version 1.0) were designed as 0 or 1. The patients had disease progression after 1 or 2 platinum-based chemotherapy regimen, and there was no chemotherapy for >3 weeks. In addition, the patients with the following clinical symptoms, such as interstitial pneumonia or pulmonary fibrosis, severe infection, intestinal paralysis or obstruction, women with pregnancy or lactation, and other serious medical conditions, were excluded. Radiological assessment of tumor size was performed once every 8 weeks by computed tomography or magnetic resonance imaging according to the RECIST. All participants have provided written informed consent in the study. The study was proved by the Ethics Committee of Peking Union Medical College Hospital and the Ethics Committee of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. All experiments were performed in accordance with relevant guidelines and regulations.

2.2. Icotinib treatment and sample collection

The patients took oral icotinib at a dose of 150 mg twice daily or 125 mg 3 times daily for a 28-continuous-day cycle followed by the intermission of 3 days until diseases progressed, unacceptable toxicity occurred, or patients refused further treatment. A series of plasma samples of each patient were collected before the treatment as the baseline and during the treatment after an overnight fasting period of about 10 h.

2.3. Quantification of plasma IIRPCs

Plasma protein complexes were isolated as our own previous study [13]. Briefly, native polyacrylamide gel electrophoresis with a gradient from 4 to 10% was used to separate plasma IIRPCs. Each run included nine test samples and one quality control sample in a single native polyacrylamide gel. The quality control sample was a mixture of 5 random patient plasma. Representative patterns of plasma IIRPCs are showed in Fig. 1. The gel bands labeled by a1, a2, a3, a4, a5, b1, b2, b3, b4, b5, b6, d1, d2, d3, e1, e2, and e3, correspond to the IIRPCs and the band labeled by the TRPC corresponds to transferrin-related protein complex. The levels of these protein complexes were quantified and their components were also identified as described previously [13].

2.4. Statistical analysis

Kaplan–Meier method was performed to determine survival. PFS is defined as the time interval between the beginning of icotinib treatment and the earliest occurrence of disease progression detected by computed tomography or magnetic resonance imaging or apparently intolerable toxicity. The time length of humoral immune and inflammation response (TLHIIR) is the time interval between the beginning of icotinib treatment and the time point at the 2-fold increase (or 0.5-fold decrease) in the IIRPC levels relative to their individual baseline. A p value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS software (version 16.0, SPSS Inc.).

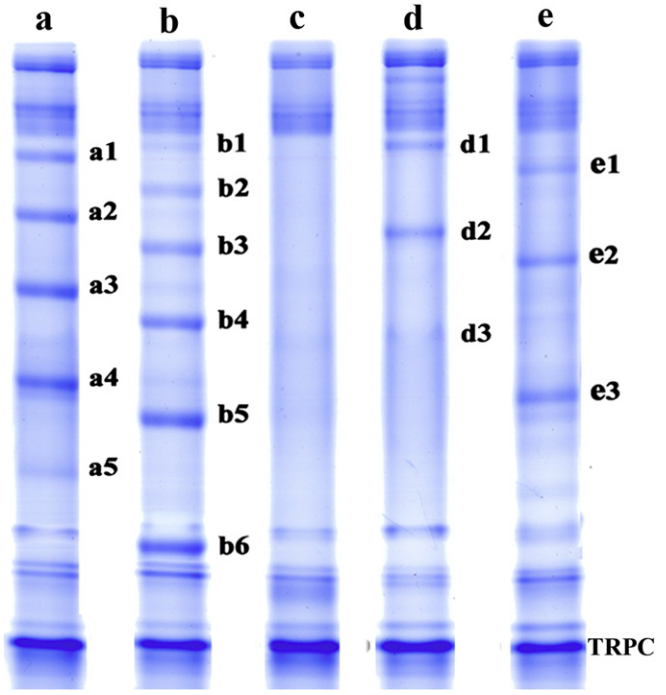

Fig. 1. Representative patterns of plasma IIRPCs. The patterns of the IIRPCs are classified into patterns a, b, c, d, and e, respectively.

3. Results

3.1. Patients

Thirty-one advanced NSCLC patients were enrolled in this study, and their clinicopathological characteristics are listed in Table 1. The mean age was 56.8 y (range 41–71 y). Eleven (35.5%) patients were male, 27 patients (87.1%) showed stage IV, nineteen patients (61.3%) had ECOG performance status of 0, 25 (80.6%) had adenocarcinoma, nine patients had EGFR mutations, 22 patients (70.9%) were never-smokers, 10 patients (32.3%) had radiotherapy and the median PFS was 34.0 weeks (95% confidential interval: 27.9 to 49.0 weeks) based on the determination of computed tomography or magnetic resonance imaging. After 2-month icotinib treatment, the response to the initial icotinib treatment was complete response in one patient, partial response in eight patients, stable disease in nineteen patients, and disease progression in 3 patients (Fig. 2A). No severe toxicity was observed and no treatment-related death occurred in this study.

3.2. Distribution of plasma protein complexes of interest

During icotinib treatment, a series of plasma samples from 31 advanced NSCLC patients were collected, and plasma protein complexes were isolated by the native polyacrylamide gel. As shown in Fig. 1, 3 patterns of plasma IIRPCs in the plasma samples collected before the treatment are observed, and 19, 8, and 4 patients were assigned as patterns (or groups) a, b and c, respectively (Table 1). For pattern a, 5 disease-specific IIRPCs were detected and labeled by a1, a2, a3, a4, and a5, respectively. For pattern b, 6 disease-specific IIRPCs were detected and