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Lung Cancer

Expression of selected gene for acquired drug resistance to EGFR-TKI in lung adenocarcinoma

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ABSTRACT

Background: Individualized treatment is an attractive challenge that may allow for more effective and safer treatment of human disease. Activating mutations in the epidermal growth factor receptor (EGFR) gene in lung adenocarcinoma are associated with a dramatic clinical response to EGFR-tyrosine kinase inhibitors (TKIs). However, patients often experience a relapse after treatment with EGFR-TKIs, even when the tumors are initially highly sensitive. However, the "whole picture" regarding acquired resistance remains unclear.
Methods: Tumor specimens were collected from 11 lung adenocarcinoma patients before and after treatment with gefitinib. The status of the EGFR and K-ras genes were investigated by PCR-based analyses. Immunohistochemistry and real-time PCR assays were used to evaluate the MET gene in terms of its tyrosine phosphorylation and amplification, respectively. The expression of HGF, PTEN, and EGR-1, and changes in the epithelial–mesenchymal transition (EMT) status including the expression of E-cadherin and gamma-catenin as epithelial markers, and vimentin and fibronectin as mesenchymal markers, were evaluated by immunohistochemistry.
Results: Seven (64%) of the gefitinib refractory tumors exhibited a secondary threonine-to-methionine mutation at codon 790 in EGFR (T790M). All of the tumors had wild type K-ras gene expression. No MET amplification was detected in any of the samples, nor was there phosphorylation of MET detected in any of the resistant samples. Neither MET gene amplification, nor the overexpression of HGF was observed in samples without the T790M mutation. A strong expression of HGF was detected in 6 of 8 specimens with the T790M mutation. Three (38%) of 8 cases showed a loss of PTEN in samples with the T790M mutation. A loss of EGR-1 was detected in 2 (29%) of 7 cases, including one tumor without PTEN. Four (57%) of 7 cases showed positive expression of phosphorylated Akt (p-Akt). A change in the EMT status between pre-and post-treatment was observed in 4 (44%) of 9 cases. In all examined samples cases, some alterations of gene or proteins were observed.
Conclusions: The current results showed that these alterations in gene or protein expression can account for all resistant mechanisms. This phenomenon suggests the existence of complicated relationships among acquired resistance-related genes.

1. Introduction

Currently, sensitive somatic mutations in the EGFR gene in lung adenocarcinoma are associated with a dramatic clinical response to EGFR-TKIs [1,2]. Thus, molecular targeted drug therapy has been promoted because the selection of patients by genetic makers can increase the therapeutic response for patients with NSCLC [3–5]. However, despite an initial response to the treatment with EGFR-TKIs in such patients, the majority of patients eventually experience a progression of the disease [2,6]. Identifying and understanding the mechanisms of treatment resistance can provide a method for blocking or reversing the mechanism of resistance.

Table 1
Summary of the patients showing acquired resistance to gefitinib.

Case	Sex	Age[a]	Smoking	Stage[b]	Stage[c]	Specimen[d]	Specimen[e]	Chemotherapy[f]	Response[g]	TTP[h] (days)
1	M	58	Never	IIIB	IIIB	Primary lung tumor	Pulmonary metastasis	Yes	PR	191
2	M	55	Never	IIIB	IIIB	Primary lung tumor	Pulmonary metastasis	No	PR	174
3	F	54	Never	IB	IIIB	Primary lung tumor	Lymph node metastasis	Yes	SD	368
4	F	70	Never	IA	IA	Primary lung tumor	Liver metastasis	Yes	PR	60
5	F	65	Current	IIIB	IIIB	Lymph node	Pleural effusion	No	PR	110
6	M	53	Current	IIIA	IIIA	Primary lung tumor	Lymph node metastasis	Yes	PR	352
7	F	84	Never	IB	IIB	Primary lung tumor	Pleural effusion	No	PR	295
8	F	57	Never	IIIA	IIA	Primary lung tumor	Lymph node metastasis	No	SD	210
9	F	76	Never	IV	IV	Primary lung tumor	Primary lung tumor	No	SD	221
10	F	85	Never	IIIA	IIIA	Primary lung tumor	Skin metastasis	No	CR	210
11	F	52	Never	IIIB	IIIB	Lymph node	Lymph node metastasis	No	PR	233

[a] Age at beginning of gefitinib therapy.
[b] Clinical stage.
[c] Stage at first presentation.
[d] Analyzed specimen of the tumor before treatment with gefitinib.
[e] Specimens at post-treatment.
[f] Previous chemotherapy.
[g] Response to gefitinib.
[h] Time to progression after gefitinib therapy.

Explanations for the EGFR-TKI resistance include the T790M mutation in exon 20 of EGFR, MET amplification, overexpression of HGF, and changes in the EMT status [6–10]. We also recently reported that gefitinib-resistant cell lines showed a marked down-regulation of PTEN expression and increased Akt phosphorylation. Furthermore, nuclear translocation of the EGR1 transcription factor, which regulates PTEN expression, was shown to be suppressed in resistant clones and restored in their revertant clones [11]. However, the "whole picture" regarding acquired resistance remains unclear, and few studies have so far investigated the resistance-related genes in EGFR-TKI resistant specimens from a translational viewpoint. Therefore, a detailed study using matched specimens from both pre- and post-treatment is essential. This study was a retrospective analysis of the acquired resistance-related gene profile in refractory tumors (recurrent and metastatic) after an initial response to treatment that was performed to elucidate the mechanism(s) underlying acquired resistance to EGFR-TKIs. To our knowledge, this is the first comprehensive analysis of the acquired resistance-related gene profile in such pre- and post-treatment study.

2. Materials and methods

2.1. Patients and their characteristics

The characteristics of the 11 patients are listed in Table 1. There were 3 male and 8 female patients. Nine patients were non-smokers and 2 patients were current smokers. The tumor stage was classified according to the International Union against Cancer tumor-node-metastasis classification of malignant tumors [12]. Eight patients from 1995 to 2007 developed recurrent disease after surgery for primary tumors. Three patients were non-surgical cases (cases 5, 9, and 11). Therefore, the pathological stage was adopted for the surgical cases, and the clinical stage for the three non-surgical cases.

The tumor samples were collected from surgically resected specimens from eight primary tumors, two metastatic lymph nodes, and one from the biopsy specimen from an endobronchially invading tumor by a transbronchial biopsy. The institutional review board's approved informed consent for the use of the tumor tissue specimens was obtained either from all the patients or from the patient's legal guardians. All patients received 250 mg gefitinib per day. The treatment was continued until the disease progressed. All of the tumors were pathologically confirmed to be adenocarcinoma. Prior chemotherapy had been administered in 4 patients.

The objective response of the patients was evaluated using the response evaluation criteria in solid tumors (RECIST) criteria, and routine clinical and laboratory assessments and chest X-rays were performed biweekly and computed tomography (CT) scans were performed 1 month after the start of gefitinib and every 3 months thereafter. Imaging studies (bone scans and brain imaging) were performed every 3 months after the initiation of gefitinib treatment. Refractory tumors were obtained from lymph node metastases (4 cases), and pulmonary metastases (2 cases), and from endobronchially invading tumor, skin metastasis, and liver metastasis (patient No. 4 by autopsy). Four separate metastatic pulmonary tumors located in segments of 4, 5, 6, and 8 of the left lung were obtained from patient No. 1 by video-assisted thoracoscopic surgery [9]. Therefore, 12 gefitinib-refractory specimens were analyzed. All of the specimens were stained with hematoxylin and eosin for the histopathological diagnosis.

2.2. Analyses of EGFR and K-ras mutations

The genomic DNA was extracted from each tumor by previously described methods [1]. The EGFR mutations in exons 19–21 were examined by sequencing [1]. The K-ras mutations were investigated by PCR-based analyses [13].

2.3. Detection of MET amplification and immunohistochemical staining for phosphorylation of MET and HGF

The MET gene copy numbers were determined by real-time PCR assays. PCR was performed for each primer set in triplicate, and the mean value was calculated [13]. Amplification was defined as more than 1.31 copies, which was calculated by the mean of the MET gene copy number measured plus 2 times the standard deviation [13]. The status of MET phosphorylation was examined by IHC staining using phosphor-specific antibodies, and was evaluated using previously described methods [13]. The status of HGF was also investigated by previously described methods [13]. The positive and the negative controls were processed by the normal gallbladder harboring over expression of HGF and the exclusion of the primary antibody, respectively [13].