

Case Report

Conversion from Positive to Negative EGFR Mutation due to Clonal Selection during Long-Term Treatment with Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors: A Case Report

Masatomo Ueda^a Masashi Namba^b Kentaro Tokumo^b Tadashi Senoo^c
Wataru Okamoto^d Masami Yamauchi^b Noboru Hattori^e
Kazuhiko Sugiyama^{b, d}

^aPostgraduate Clinical Training Center, Hiroshima University Hospital, Hiroshima, Japan;

^bDepartment of Clinical Oncology, Hiroshima University Hospital, Hiroshima, Japan;

^cDepartment of Respiratory Medicine, National Hospital Organization, Kure Medical Center,

Hiroshima, Japan; ^dCancer Treatment Center, Hiroshima University Hospital, Hiroshima,

Japan; ^eDepartment of Respiratory Medicine, Hiroshima University Hospital, Hiroshima,

Japan

Keywords

Epidermal growth factor receptor mutation · Gefitinib · Erlotinib · Non-small cell lung cancer · Mechanisms of resistance

Abstract

A 77-year-old woman with postoperative recurrent non-small cell lung adenocarcinoma, which exhibited an *epidermal growth factor receptor (EGFR)* L858R mutation, was treated with gefitinib and erlotinib. Seven years after the start of treatment, the patient experienced a recurrence of malignant pleural effusion. However, 3 different genetic tests revealed that the lung adenocarcinoma cells in the pleural effusion had lost *EGFR* L858R mutation, suggesting that long-term treatment with EGFR-tyrosine kinase inhibitors (TKIs) converted *EGFR* mutation from positive to negative. The negative conversion of *EGFR* mutation as a mechanism of acquired resistance to EGFR-TKIs is considered rare and needs to be further investigated.

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Correspondence to:
Masashi Namba, nambam@hiroshima-u.ac.jp

Introduction

Patients with epidermal growth factor receptor (*EGFR*)-mutant lung cancer develop acquired resistance to *EGFR*-tyrosine kinase inhibitors (TKIs) within a year [1–3], despite an initial good response to *EGFR*-TKIs. Some possible mechanisms for acquired resistance, such as *EGFR* T790M or *MET* amplification [4], have been identified, but there are only a few reports that suggest negative conversion of *EGFR* mutation as a cause of resistance [5, 6]. Herein, we report the case of a patient with *EGFR*-mutant lung adenocarcinoma who experienced loss of *EGFR* L858R mutation after 7 years of *EGFR*-TKI therapy.

Case Report

A 77-year-old Japanese woman underwent upper lobectomy of the left lung for lung adenocarcinoma (cT1N0M0 stage IA) in 2004. In 2009, she received chemoradiotherapy (carboplatin + docetaxel, 70 Gy in 28 fractions) because of postoperative recurrence. In 2012, positron emission tomography scans showed an increasing consolidation in the right middle lobe, which indicated a recurrence of lung cancer (Fig. 1A). Genetic analysis showed that the surgically resected specimen had an *EGFR* L858R mutation, and she was treated with gefitinib (250 mg/day) and achieved a partial response.

In 2017, during treatment with gefitinib without recurrence of lung cancer, CT scans showed a mass in the stomach (Fig. 1B), which was diagnosed as a gastrointestinal stromal tumor (GIST). The patient underwent proximal gastrectomy. In March 2019, multiple low-density areas appeared and were diagnosed as liver metastases of GIST by liver biopsy (Fig. 1C, D). She was subsequently referred to our hospital for chemotherapy.

In April 2019, imatinib (300 mg/day) was started for multiple liver metastases of GIST while remaining on gefitinib (250 mg/day) for lung cancer. In July 2019, gefitinib was replaced with erlotinib (100 mg/day) because she presented with grade 3 (Common Terminology Criteria for Adverse Events version 5.0) diarrhea following an interaction between gefitinib and imatinib. In January 2020, CT scans showed increased right lung consolidation and pleural effusion, although multiple liver metastases of GIST continued to shrink (Fig. 1E, F), and the patient's carcinoembryonic antigen level increased. She had progressive lung cancer, although multiple liver metastases of GIST were controlled. Erlotinib was discontinued, and gefitinib was re-started because the patient refused to receive cytotoxic agents. She continued gefitinib and imatinib treatment without any side effects. However, lung cancer has progressed, and fatigue and anorexia have worsened.

We suspected the appearance of *EGFR* T790M mutation and submitted a plasma *EGFR* mutation test (Cobas[®] *EGFR* mutation test version 2.0) in March. Both *EGFR* L858R, which was originally positive, and T790M mutations were negative. To eliminate the possibility of false-negative results, we submitted pleural fluid that was pathologically diagnosed as adenocarcinoma cells for an *EGFR* mutation test (peptide nucleic acid-locked nucleic acid polymerase chain reaction [PCR] clamp). However, *EGFR* mutations were not detected. Subsequently, we prepared a cell block from the pleural fluid specimens (Fig. 2) and submitted it for an *EGFR* mutation test (Cobas[®] *EGFR* mutation test version 2.0), but the results were similar (Table 1).

The patient's performance status decreased, and she and her family wanted the best supportive care. Therefore, she discontinued both gefitinib and imatinib and died approximately 1 month later.

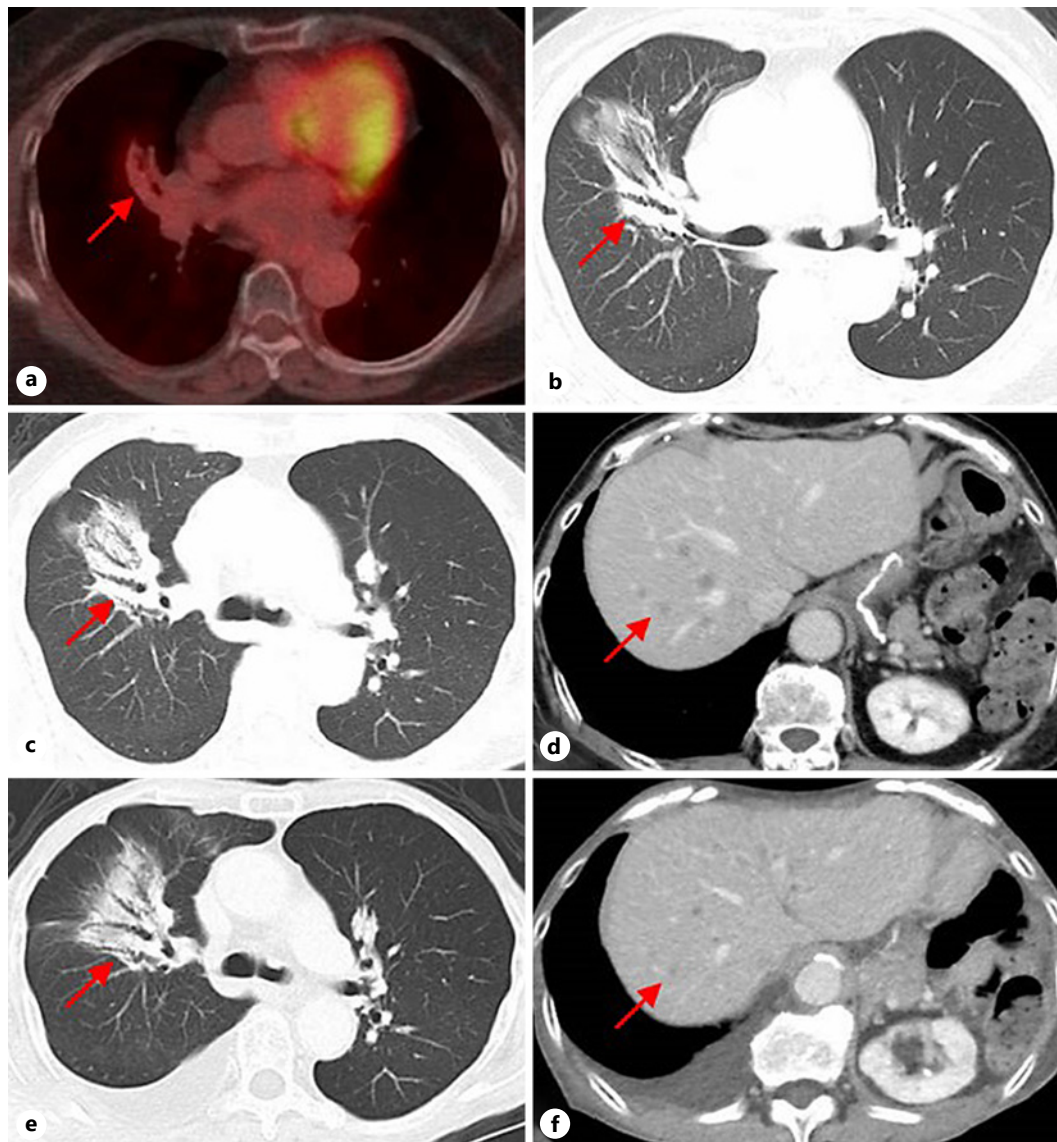


Fig. 1. Lung cancer and GIST in CT images. **A** PET scans showed bronchocentric consolidation on the right middle lobe in 2012. **B** Consolidation on the right middle lobe had not changed since 2012, when she was diagnosed with GIST in 2017. In April 2019, consolidation on the right middle lobe had not changed (**C**), and multiple low-density areas of the liver appeared (**D**). In January 2020, consolidation on the right middle lobe had increased and pleural effusion emerged (**E**), while liver metastases of GIST had continued to shrink (**F**). GIST, gastrointestinal stromal tumor; PET, positron emission tomography.

Discussion

This patient was treated with EGFR-TKI for postoperative recurrence of *EGFR* L858R-mutant lung adenocarcinoma and maintained a partial response for approximately 7 years. Finally, it became refractory to EGFR-TKI; thus, we suspected the emergence of the T790M mutation and performed the EGFR mutation test 3 times with different modalities. However, *EGFR* mutation was negative.

We performed 3 different EGFR mutation tests after the patient became refractory to EGFR-TKIs. Currently, there are 2 main types of EGFR mutation tests: plasma-based tests

Fig. 2. Cell block of adenocarcinoma cells from pleural fluid specimens. Immunostaining showed that thyroid transcription factor-1 and napsin A were positive, P40 and CK5/CK6 were negative, and programmed death-ligand 1 expression (tumor proportion score) was 0%. CK, cytokeratin.

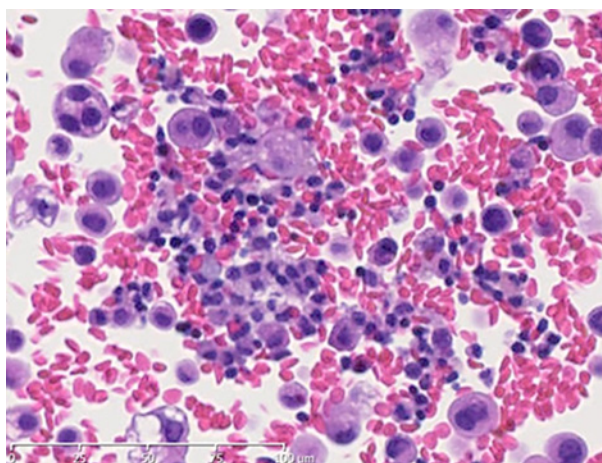


Table 1. EGFR mutation tests performed during the course of the disease

	Daytime	Specimen	Method	EGFR mutation
Before EGFR-TKI administration	January 2004	Tumor tissue surgically resected	PCR – Invader	L858R
After EGFR-TKI administration	March 2020	Plasma	Cobas® EGFR mutation test version 2.0	All negative
	April 2020	Pleural fluid	Peptide nucleic acid-locked nucleic acid PCR clamp	All negative
		Cell block made from the pleural fluid specimens	Cobas® EGFR mutation test version 2.0	All negative

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PCR, polymerase chain reaction.

and tissue-based tests. It has been reported that the sensitivity of the Cobas plasma test (Cobas® EGFR mutation test version 2.0) is approximately 76.7% compared to the Cobas tissue test (Cobas® EGFR mutation test version 2.0) and approximately 90% compared to the next-generation sequencing using plasma samples [7]. Therefore, if the plasma test is negative, some cases will be re-tested with a tissue sample to rule out a false-negative result. The Cobas tissue test, in which the minimum detection sensitivity for the *EGFR* L858R mutation is approximately 4% [7], was reported to be slightly more accurate than the PCR clamp test [8]. Thus, we performed a Cobas tissue test using cell blocks after performing the PCR clamp test as a third test. Considering that the 3 different tests were all negative, we concluded that the lung adenocarcinoma at the time of recurrence was truly negative for *EGFR* mutation.

In our case, conversion from *EGFR* L858R mutation to wild-type *EGFR* was considered to be the main cause of disease progression to EGFR-TKIs. The main mechanisms of resistance to EGFR-TKIs are *EGFR* T790M mutation, *MET* amplification, *HER2* amplification, and small cell lung cancer transformation [4, 5, 9]. Although we did not investigate other resistance mechanisms, such as *MET* amplification, except *EGFR* T790M, it is certain that *EGFR* mutations are retained when these known acquired resistances occur [6]. Therefore, we considered that the negative conversion of *EGFR* mutations may play a critical role in disease progression.

Table 2. Case series of loss of *EGFR* mutation

Age and sex	Pathology	<i>EGFR</i> mutation	Anticancer drugs used until loss of <i>EGFR</i> mutation	Time to treatment failure	References
49 yr, male	Adenocarcinoma	L858R	Gefitinib	7 months	[6]
34 yr, female	Large cell carcinoma	Exon19 deletion (L747-T751)	Cisplatin + docetaxel Pemetrexed Gefitinib (250 mg/body) Erlotinib (150 mg/body) Irinotecan + amrubicin	3 months	[11]
30 yr, female	Adenocarcinoma	L858R, Q787Q	Cisplatin + pemetrexed Erlotinib (150 mg/body)	34 months	[12]
87 yr, male	Adenocarcinoma	Exon19 deletion	Gefitinib (250 mg/body)	9 months	[13]
89 yr, female	Adenocarcinoma	L858R	EGFR-TKI (drug unknown)	9 months	[14]
77 yr, male	Adenocarcinoma	L858R	Gefitinib (250 mg/body) Erlotinib (100 mg/body)	94 months	This case

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

Approximately 15–20% of acquired resistance remains unknown; negative conversion of *EGFR* mutations has been reported in a small number of cases [5, 9, 10]. Ji et al. [6] reported that 1 patient showed loss of *EGFR* mutation in a pathological review of tissues from 25 patients who responded well to gefitinib and later became refractory to treatment. To the best of our knowledge, there are 5 case reports with detailed medical records [6, 11–14] (Table 2). The duration of EGFR-TKI treatment ranged from 3 to 34 months in these reports, and no other reports have shown negative conversion of *EGFR* mutations after long-term EGFR-TKI exposure, as in our case.

In our case, it is possible that long-term treatment with EGFR-TKIs resulted in selective growth of EGFR mutation-negative tumor cells due to genetic heterogeneity in the tumor tissue. Bai et al. [10] reported that there was intratumor heterogeneity of *EGFR* mutations in approximately 62% of *EGFR* mutation-positive non-small cell lung carcinoma. Furthermore, chemotherapy resulted in negative conversion of *EGFR* mutations. Therefore, in this case report, long-term treatment with EGFR-TKIs eliminated *EGFR* mutation-positive cells, but only *EGFR* mutation-negative cells survived selectively, leading to recurrence in the form of increased pleural effusion. In contrary, the possibility of another lung cancer without *EGFR* mutation should also be considered as 7 years have passed since the start of EGFR-TKIs; however, it seems that this possibility is low because the foci in the lung fields at the time of treatment initiation and recurrence were the same.

In conclusion, this case report demonstrated that the conversion from positive to negative *EGFR* mutation is one of the causes of acquired resistance to EGFR-TKIs. Clonal selection due to long-term administration of EGFR-TKIs for lung cancer with genetic heterogeneity was considered to be the cause of the negative conversion. The mechanism of negative conversion of EGFR mutations needs to be further investigated.

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Statement of Ethics

The study is exempt from ethics committee approval due to only one case report. Written informed consent was obtained from the patient before her death for publication of this case report and any accompanying images.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

All authors contributed to the conception and design of the study. Material preparation and data collection were performed by Masatomo Ueda and Masashi Namba, respectively. The first draft of the manuscript was written by Masatomo Ueda and Masashi Namba. All authors commented on the previous version of the manuscript and approved the final manuscript.

Data Availability Statement

All the data that support the findings of this study are included in this manuscript.

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