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CASE REPORT

Pitfalls in diagnosis with the use of circulating tumor-derived epidermal growth factor receptor mutations in lung cancer harboring pretreatment T790M

Daiki Ogawara[1], Hiroshi Soda[1], Takayuki Suyama[1], Masataka Yoshida[1], Tatsuhiko Harada[1], Yuichi Fukuda[1] & Hiroshi Mukae[2]

1 Department of Respiratory Medicine, Sasebo City General Hospital, Nagasaki, Japan
2 Department of Respiratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

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Correspondence
Hiroshi Soda, Department of Respiratory Medicine, Sasebo City General Hospital, 9-3 Hirase, Sasebo, Nagasaki 857-8511, Japan.
Tel: +81 956 24 1515
Fax: +81 956 22 4641
Email: h-souda@hospital.sasebo.nagasaki.jp

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Abstract

The circulating tumor DNA (ctDNA) assay has recently been approved for the selection of EGFR-tyrosine kinase inhibitors as first-line treatment in lung cancer. However, it remains to be determined whether this assay can detect all complex *EGFR* mutations within a single tumor. We report a case of an elderly woman with stage IV lung adenocarcinoma, in which *EGFR* mutation assays detected L858R and pretreatment T790M from a tissue biopsy. In contrast, the circulating tumor DNA assay detected L858R, but not pretreatment T790M in the plasma, regardless of the fact that similar amounts of each mutation were present in the biopsy specimen. Treatment with afatinib was not effective, but subsequent treatment with osimertinib remarkably regressed the tumor. Our findings indicate that physicians should accurately evaluate EGFR-tyrosine kinase inhibitor-insensitive mutations using tissue samples in the first-line setting, even when L858R and exon 19 deletions are detected in the plasma.

Introduction

Both the therascreen (Qiagen, Hilden, Germany) and cobas (Roche, Basel, Switzerland) assays used to determine circulating tumor DNA (ctDNA) of L858R and exon 19 deletions have been approved for use with first-generation EGFR-tyrosine kinase inhibitors (TKIs) in the European Union and the United States. Multiple *EGFR* mutations are sometimes observed in a single tumor.[1,2] Mutations resistant to EGFR-TKIs, such as T790M and exon 20 insertions, are in part derived from pre-existing subclones before treatment.[3] The anti-tumor activity of EGFR-TKIs varies with the kind of *EGFR* mutation, and the results of ctDNA assay may thus influence the choice of EGFR-TKI.[4,5] However, whether this assay can detect all components of complex *EGFR* mutations in the same tumor is undetermined. We herein report a case of lung adenocarcinoma containing both L858R and pretreatment T790M, known as de novo T790M, in which the ctDNA assay detected only L858R. Additionally, treatment with the third-generation EGFR-TKI osimertinib, but not the second-generation EGFR-TKI afatinib, regressed this lung cancer containing pretreatment T790M.

Case report

A 65-year-old, never-smoking woman was referred to our hospital for evaluation of a lung nodule that was detected on chest radiography. A chest computed tomography scan showed a 34 mm solid nodule in the right lower lobe with multiple small nodules in both lungs, evidence of lymphangitic carcinomatosis in the right lower lobe, and enlargement of the subcarinal and left supraclavicular lymph nodes (Fig 1a,b). Transbronchial biopsy revealed poorly differentiated adenocarcinoma, with immunostaining positive for TTF-1 and negative for P40. She was eventually diagnosed with stage IV (cT4N3M1a) lung adenocarcinoma with pulmonary metastases by systemic survey including positron emission tomography and magnetic resonance imaging. The cobas *EGFR* mutation test v2 conducted using the biopsy specimen detected the L858R mutation and the

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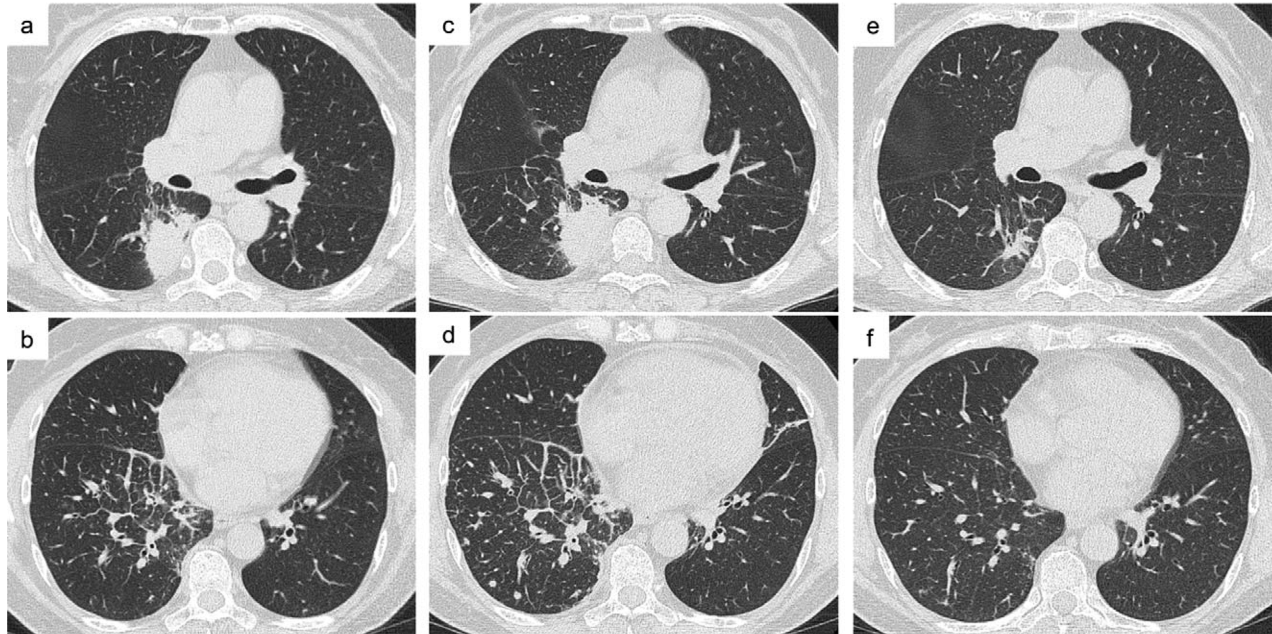

Figure 1 Chest computed tomography scans of the patient with lung adenocarcinoma. Before treatment with afatinib, (**a**) a 34 mm tumor and (**b**) interlobular septal thickening were observed in the right lower lobe of the lung. After four weeks of afatinib therapy, and before treatment with osimertinib, (**c**) the lung tumor had enlarged and (**d**) new small metastatic nodules had appeared. Six months after treatment with osimertinib, (**e**) the lung tumor had remarkably regressed, and (**f**) the metastatic nodules and interlobular septal thickening were no longer observed.

pretreatment T790M mutation, whereas the same test conducted using the plasma sample detected only L858R (Table 1). In order to semi-quantitate the amount of each *EGFR* mutation in the tissue specimen, the PCR-invader method (BML Inc., Tokyo, Japan) was used. As previously reported, the amount of each *EGFR* mutation was estimated as follows: (fluorescence of the specimen with detection probe for mutation sequences) – ([fluorescence of the normal control with the same probe] × 2).[6] The relative fluorescent intensity was 3.3×10^4 units for L858R and 2.8×10^4 units for pretreatment T790M, suggesting that the amount of each mutation did not differ considerably. The patient received afatinib at a dose of 40 mg once daily. However, the lung lesions enlarged, and new lung metastases appeared four weeks after the initiation of therapy (Fig 1c,d). Serum cytokeratin 19 fragment (CYFRA21-1) levels increased from 3.6 ng/mL to 5.4 ng/mL (reference value < 3.5 ng/mL). The treatment regimen was thus subsequently changed to osimertinib, at a dose of 80 mg once daily. The tumor began to reduce in size one week later, and a partial response was achieved six weeks later. The serum CYFRA 21-1 levels decreased to 1.3 ng/mL. The patient's lung cancer has remained progression-free for seven months (Fig 1e,f). Written informed consent for the publication of this case report was obtained from the patient.

Discussion

In this case study, conventional ctDNA assay was able to detect the L858R mutation, but not the pretreatment T790M mutation using a plasma sample. There are various mechanisms by which DNA from cancer cells is released into the bloodstream, including cellular events such as apoptosis, necrosis, and even direct secretion.[7] There are two possible reasons for the discrepancy in the detection of *EGFR* mutations by the methods used in this study. Either there were a smaller number of cancer cells containing the pretreatment T790M mutation within the tumor, or there was less release of DNA from such cells. Arguing against the former possibility, however, the semi-quantitative DNA analysis of the biopsy specimen in this study indicated that the representation of L858R and pretreatment T790M mutations was similar.

Another possible factor for this discrepancy is that reduced DNA release may reflect the cell biology. A preclinical study using lung cancer cell lines revealed that cell growth is slower in subclones with post-treatment acquired T790M than parental cells.[8] Accordingly, detection in plasma samples has been reported to be lower for acquired T790M than for L858R or exon 19 deletions.[9–15] The sensitivity of ctDNA assays ranges from 71% to 87% for exon 19 deletions and L858R, whereas the sensitivity is 61% to 81% for post-treatment acquired T790M (Table 2). These

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Table 1 Analysis of *EGFR* mutations in tissue and plasma samples

	Tissue		Plasma
EGFR mutations	cobas mutation test v2	Relative fluorescence intensity with PCR-invader method ($\times10^4$ units)	cobas mutation test v2
Exon 18 G719X	ND	ND	ND
Exon 19 deletions	ND	ND	ND
Exon 20 S768I	ND	ND	ND
Exon 20 insertions	ND	NE	ND
Exon 20 T790M	Detected	2.8	ND
Exon 21 L858R	Detected	3.3	Detected
Exon 21 L861Q	ND	ND	ND

ND, not detected; NE, not evaluated.

findings suggest that DNA containing acquired T790M was less prone to being shed into the bloodstream as a result of the indolent cell growth of T790M subclones.[16] However, little is known about the discrepancy in the detection of pretreatment T790M between ctDNA and tissue DNA and the relationship between pretreatment T790M and post-treatment acquired T790M. Further studies are required to clarify the cell biology and clinical significance of pretreatment T790M.

Another important finding from this study is that osimertinib treatment was shown to have anti-tumor activity against a lung cancer harboring both L858R and pretreatment T790M mutations, whereas afatinib was not effective. The in vitro anti-tumor activity of EGFR-TKIs varies with the kind of *EGFR* mutation.[4,5] Clinically available EGFR-TKIs, with the exception of osimertinib, are all less effective against cell lines carrying T790M, while the existence of more than 25% of cells harboring T790M has been shown to strongly decrease the in vitro anti-tumor activity of erlotinib.[8] In clinical trials, afatinib has shown weak activity in lung cancer patients with pretreatment T790M. In 14 lung cancer patients carrying pretreatment T790M, the overall response rate and median progression free-survival were 14.3% and 2.9 months, respectively.[17] In contrast, osimertinib shows promising results in patients carrying the mutation. A phase I expansion study of osimertinib has revealed a partial response in all five lung cancers harboring pretreatment T790M, with the duration of response > 12 months.[18]

In conclusion, to the best of our knowledge, this article is the first to report that the results of the conventional ctDNA assay may not reflect pretreatment T790M of complex *EGFR* mutations within a lung tumor. In order to select the optimal first-line therapy for lung cancer

Table 2 Performance of ctDNA assays for detection of post-treatment *EGFR* mutations in lung cancer as reported in the literature

EGFR mutations	Methods	Number of samples	Sensitivity (%)	Specificity (%)	References
Exon 19 deletions	Cobas	551	85	98	Jenkins[9,10]
L858R			76	98	
Acquired T790M			61	79	
Exon 19 deletions	Cobas	110	71	100	Karlovich[11]
L858R			78	100	
Acquired T790M			64	98	
Exon 19 deletions	Cobas	72	82	97	Thress[12]
L858R			87	97	
Acquired T790M			73	67	
Exon 19 deletions	BEAMing	216	82	98	Oxnard[13]
L858R			86	97	
Acquired T790M			70	69	
Exon 19 deletions	BEAMing	72	82	97	Thress[12]
L858R			87	97	
Acquired T790M			81	58	
Exon 19 deletions	ddPCR	54	81	100	Sacher[14]
L858R			78	100	
Acquired T790M			77	63	
L858R/exon 19 deletions	ddPCR	41	76	88	Takahama[15]
Acquired T790M			65	70	

BEAMing, beads, emulsion, amplification, and magnetics; ctDNA, circulating tumor DNA; ddPCR, droplet digital-PCR.

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patients, physicians should accurately evaluate the status of *EGFR* mutations using tumor tissue specimens, even when L858R or exon 19 deletions are detected in the plasma sample.

Disclosure

No authors report any conflict of interest.

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