CASE REPORT

Non-responsiveness to Gefitinib in a patient with lung adenocarcinoma having rare *EGFR* mutations S768I and V769L

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Summary

Mutations in the tyrosine kinase domain of *epidermal growth factor receptor (EGFR)* are associated with clinical responsiveness to EGFR tyrosine kinase inhibitors (EGFR-TKIs) in patients with non-small cell lung cancers (NSCLCs). However, certain rare *EGFR* mutations including S768I are reported to confer less *in-vitro* sensitivity to gefitinib, an EGFR-TKI, than major mutations such as exon 19 deletions and L858R, and even the wild-type counterpart. Here, we report the first case of adenocarcinoma of the lung in which the patient had rare mutations S768I and V769L and was treated with gefitinib. Disease progressed during six weeks of treatment. This case suggests that in vitro sensitivity to gefitinib correlates with distinct clinical responsiveness to gefitinib in various types of *EGFR* mutations.

Key words

epidermal growth factor receptor (EGFR); mutation; non-small cell lung cancer (NSCLC); gefitinib; chemotherapy; first-line therapy

1.Introduction

Recently, somatic mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR) gene were reported to exist in a subset of non-small cell lung cancers (NSCLCs).[1-3] These mutations are closely correlated with favorable clinical responsiveness to EGFR-tyrosine kinase inhibitors (EGFR-TKIs). It was also demonstrated that two major types of mutations, exon 19 deletions and L858R, enhanced TK activity in response to EGF and increased the *in-vitro* sensitivity to an EGFR-TKI, gefitinib. In addition to these two types of mutations, various rare mutations have been identified;[4-10] however, in *in-vitro* studies, some of these mutations (including S768I) conferred less sensitivity to gefitinib than the two major types of mutations, and even its wild-type counterpart.[11] Because of lower frequency of these novel mutations, clinical information of their relationships with drug responsiveness is very limited so far. We herein report a case of a patient with adenocarcinoma of the lung who exhibited two such rare mutations, S768I and V769L, and was treated with gefitinib.

2. Case report

A 61-year-old male patient was admitted to our hospital in May 2005 after undergoing resection of a metastatic brain tumor from lung adenocarcinoma. He was a current heavy smoker who had 70 pack-year of smoking index. His chest CT scan revealed a 36-mm nodule in the right S^1 with multiple miliary metastasis in both lung fields (Fig. 1a,b). He also exhibited contralateral mediastinal lymphadenopathy, bilateral adrenal gland metastasis, and sacroiliac joint metastasis. His disease was accordingly staged as cT4N3M1, stage IV. Thrombocytopenia was also evident (platelet count < 100,000 / ul) and bone marrow aspiration revealed myelodysplastic syndrome. Taking the patient's general condition and opinion into account, we offered him gefitinib treatment at an oral dose of 250 mg daily as first-line therapy.

The nine-week Gefitinib administration period included a

three-week interruption, during which the patient underwent radiation therapy for residual brain tumor. However, despite over six weeks of gefitinib administration, chest and abdominal CT revealed enlargement of the primary lesion and pulmonary and adrenal metastases. These lesions were therefore thought to be resistant to gefitinib and the response was evaluated as progressive disease according to the RECIST criteria (Fig. 1c,d). However, as his functional status had not declined and he wanted chemotherapy, combination chemotherapy with carboplatin (AUC 5, day 1) and weekly paclitaxel (70 mg/m^2, day 1, 8, 15) was administered. After two cycles of this chemotherapy, remarkable shrinkage of both primary and metastatic lesions was observed on chest and abdominal CT, leading to partial response (PR) (Fig. 1e,f). He continues to receive chemotherapy as of February 2006.

We obtained written informed consent to investigate *EGFR* mutations and *EGFR* copy number in his NSCLC tissue. Genomic DNA was isolated from two sections of paraffin-embedded tissue of the resected brain metastasis using a DNeasy Tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Exons 18 to 21 were amplified using primers and conditions previously reported.[2] PCR products were purified using a PCR purification kit (Qiagen) and sequenced directly using an Applied Biosystems BigDye Terminator kit v3.1 with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). These sequencing reactions were performed in both forward and reverse directions from two independent amplification products. Sequencing analysis revealed that there were no major mutations such as G719X in exons 18, exon 19 deletions, and L858R in exon 21. However, double point mutations of S768I and V769L (AGCGTG to ATCTTG in sense direction, Fig. 2a and CACGCT to CAAGAT in antisense direction, Fig. 2b) were detected in exon 20. *EGFR* copy number per cell was investigated by FISH using the LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis, Abbott Laboratories, IL, USA), according to a published protocol and

classification.[12] This case had *EGFR* amplification, since more than 15 copies of *EGFR* per cell in ≥ 10% of analyzed cells were observed (Fig. 3).

3. Discussion

To the best of our knowledge, this is the first reported case of a patient with the rare mutations S768I and V769L treated with gefitinib. The relationship between *EGFR* mutations and sensitivity to EGFR-TKI in NSCLCs remains controversial.[1-10,13] Although multiple studies have shown striking correlation between these factors,[1-3,5,8-10] a correlative study in a large clinical phase III trial, BR21, showed no significant association of *EGFR* mutations with responsiveness to erlotinib or with survival after this agent was administered.[13] One possible reason for such a discrepancy could be that different mutations have different effects on response to EGFR-TKIs. In most of the former studies, over 90% of the mutations were exon 19 deletions and L858R,[1-10] whereas half of the mutations consisted of other sporadic and rare mutations in the latter study.[13]

S768I, a mutation in exon 20 of *EGFR*, is one of those rare mutations. Retrospective analyses of *EGFR* mutations using surgical specimens that have demonstrated such mutations have included that by Huang *et al*.[5] (revealing S768I) and that by Kosaka *et al*.[4] (demonstrating S768I and V769L). However, the clinical responsiveness of EGFR-TKIs to these mutations has not been shown.

In *in-vitro* studies, various mutations were shown to have the distinctive phosphorylation patterns of several C-terminal Tyrosine (Tyr) residues of *EGFR* and different sensitivities to gefitinib when they were stably transfected to a NSCLC cell line.[11,14] Some of these mutations, including S768I, are hyperphosphorylated on the Tyr 1045 residue, which is known to recruit Cbl to EGFR and to lead to Cbl-mediated receptor multi-ubiquitination, and are refractory to EGF-induced ubiquitination and degradation.[15-17] Gefitinib causes less growth-suppressive effects on cells expressing these mutations

than those expressing exon 19 deletions or L858R mutations and even those expressing the wild type counterpart.[11] Although the role of V769L mutations in the responsiveness to gefitinib remains to be examined *in vitro*, clinical responsiveness of the present case with S768I mutation was consistent with the previously-shown *in-vitro* biological phenotype and gefitinib sensitivity of this mutation.

The observed EGFR gene amplification in this case is intriguing because increased gene copy number of *EGFR* are reported to be associated with higher response rate and better survival to EGFR-TKI.[12,13] Taken together with *in-vitro* finding that S768I mutation shows less gefitinib sensitivity than wild-type[11], amplification of the mutant allele with such low sensitivity, S768I, may not overcome resistance to gefitinib.

Since this is a case report, multiple factors other than two *EGFR* mutations S768I and V769L in this case might be associated with resistance to gefitinib. One of the possible factors is his smoking status. *K-ras* mutations are associated with heavy smoking and gefitinib resistance.[18] Althouth his *K-ras* status has not been examined, he might have the *K-ras* mutation since he was a heavy smoker. Another possible factor is the presence of *HER2 (ErbB2)* mutations. Although HER2 mutations in lung cancer are rare, they have also been reported to be associated with the resistance to another EGFR-TKI, Erlotinib.[19] Heterogeneity of the tumor might also affect the clinical response, since his mutational analysis was done using a surgical sample from one of the metastatic sites.

4. Conclusion

Despite the limitations of a case report, this case is an example of a patient with different *EGFR* mutations who demonstrated distinctive clinical responsiveness to EGFR-TKIs and in whom *in-vitro* sensitivities correlated with clinical responsiveness to EGFR-TKIs. Additional functional and clinical analyses for various *EGFR* mutations are accordingly warranted.

5. Conflict of interest statement

None declared.

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Figure Legends

Figure. 1

Chest CT before gefitinib administration shows a 36 mm nodule in the right S^1 (a) with miliary metastases in both lung fields (b). After six weeks of gefitinib administration, enlargement of both the primary (c) and miliary metastatic lesions (d) was observed. After combination chemotherapy, CT shows remarkable shrinkage of both the primary (e) and miliary metastatic lesions (f).

Figure. 2

(a) The forward sequence of exon 20 of *EGFR* showed mutations of 2303 G to T and 2305 G to T. (b) The reverse sequence of the same site shows mutations of 2303 C to A and 2305 C to A. Both results indicate amino acid substitutions S768I and V769L.

Figure. 3

FISH was performed with the EGFR (red)/CEP7 (green) probe. Total nuclear DNA was stained with 4'6'-diamidino-2-phenylindole (Blue). Increased red clusters in each nucleus showed *EGFR* amplification.

Fig. 1

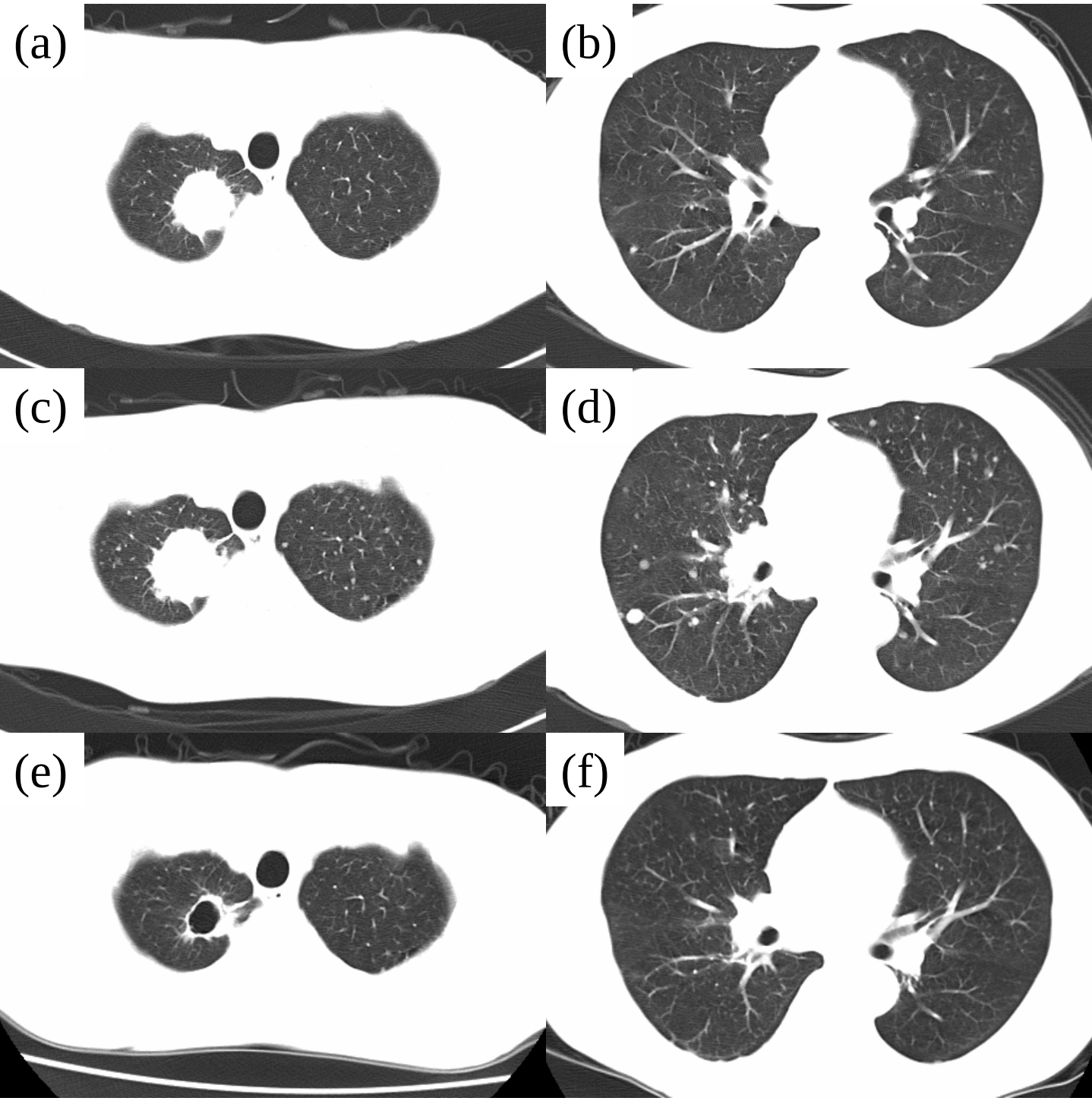
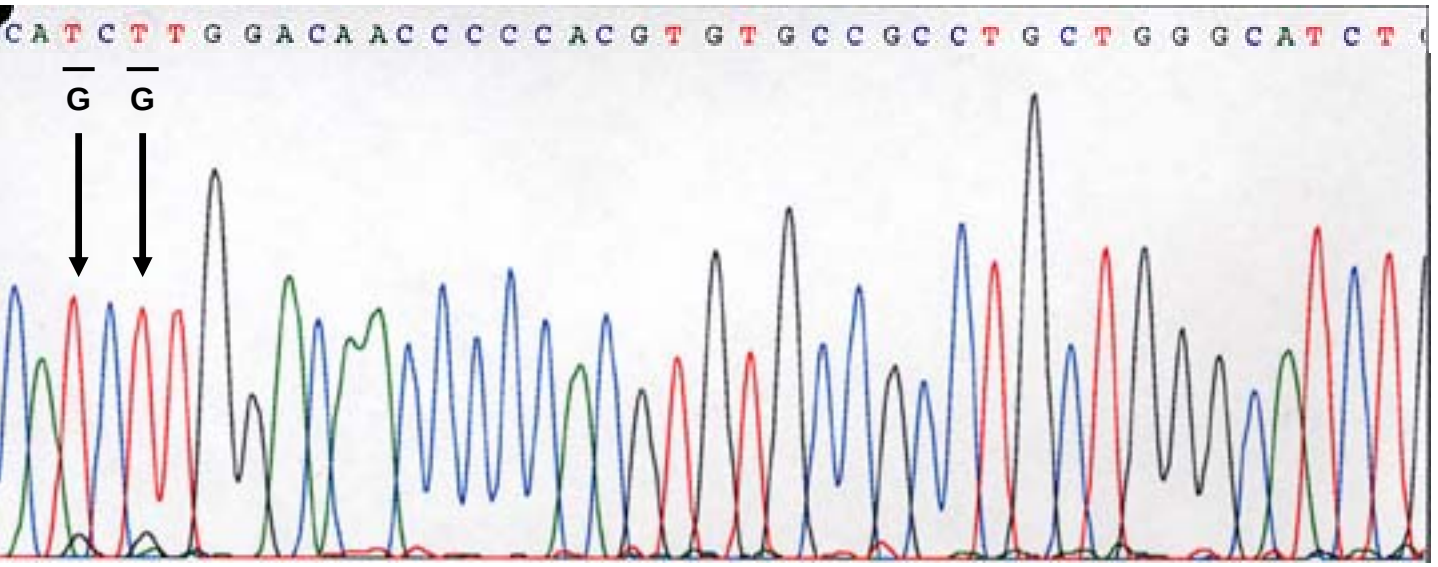


Fig. 2

(a)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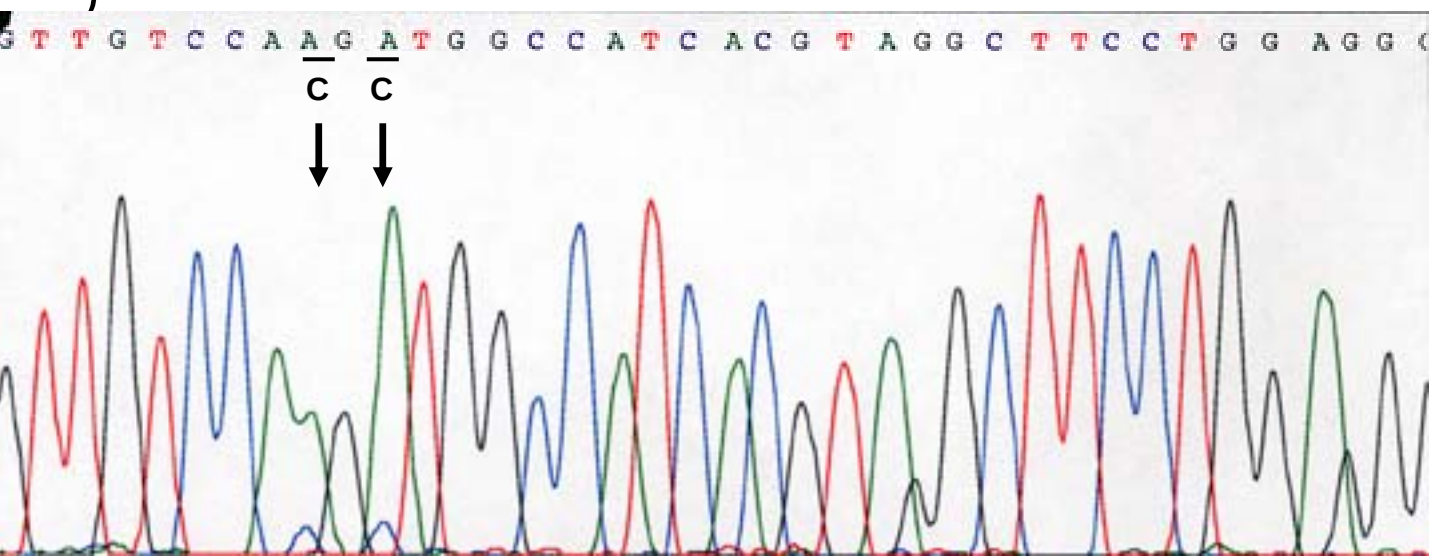
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Fig. 3