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Case report: Durable response to afatinib in a patient with lung cancer harboring two uncommon mutations of *EGFR* and a *KRAS* mutation

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ABSTRACT

Comprehensive genomic profiling for non–small cell lung cancer (NSCLC) is likely to identify more patients with rare genetic alterations including uncommon epidermal growth factor receptor gene (*EGFR*) mutations. It remains unclear how such patients should be treated, however. We here report a case of NSCLC positive for two uncommon mutations of *EGFR* and a *KRAS* mutation, including its treatment with the second-generation EGFR tyrosine kinase inhibitor (TKI) afatinib. Tumor specimen obtained by a NSCLC patient with no smoking history was analyzed by next-generation sequencing. Comprehensive genomic profiling revealed that the patient harbored the *EGFR* mutations G719C and S768I as well as the E49K mutation of *KRAS*. Treatment with afatinib was clinically effective as confirmed by PET-CT scans of bone metastases and by a marked decrease in the serum concentration of carcinoembryonic antigen. Afatinib was the most effective among seven EGFR-TKIs tested in inhibiting the growth of Ba/F3 cells expressing EGFR(S768I), showing an efficacy similar to that apparent with cells expressing the common EGFR mutant L858R, whereas first- and third-generation EGFR-TKIs were markedly less effective against EGFR(S768I) than against EGFR(L858R). These data suggest that EGFR-TKIs differ in their activity toward cells expressing EGFR(S768I) in vitro. Consistently, afatinib was clinically effective for the treatment of NSCLC harboring G719C and S768I mutations of *EGFR*. Further studies are warranted to determine the most appropriate EGFR-TKI for treatment of NSCLC harboring uncommon *EGFR* mutations.

1. Introduction

First-generation tyrosine kinase inhibitors (TKIs) for the epidermal growth factor receptor (EGFR), including gefitinib and erlotinib, have been found to markedly improve overall survival of patients with non–small cell lung cancer (NSCLC) positive for so-called common *EGFR* mutations. These common mutations comprise deletions in exon 19 and the Leu858Arg (L858R) point mutation in exon 21, and they account for ~90% of all *EGFR* mutations [1]. Newer EGFR-TKIs—such as the second-generation drugs afatinib, neratinib, and dacomitinib as well as the third-generation agents osimertinib, rociletinib, and ASP8273—have also shown therapeutic efficacy in patients with these common mutation [2–5]. Although recent data have indicated that afatinib is also active in NSCLC patients with certain types of uncommon *EGFR* mutation [6], the relation between such mutations and the response to EGFR-TKIs remains unclear because of the relative rarity of these genetic changes.

Mutations of *KRAS* are present in ~15% to 20% of patients with lung adenocarcinoma [7]. Given that mutations of *EGFR* and *KRAS* are essentially considered to be mutually exclusive in NSCLC, it is unclear how patients found to have both *EGFR* and *KRAS* mutations should best be treated. We now report the case of a NSCLC patient positive for both two uncommon mutations of *EGFR*—G719C in exon 18 and S768I in exon 20—and an E49K mutation of *KRAS* who was treated with afatinib. Furthermore, we examined the sensitivity of cells harboring the S768I mutation of *EGFR* to first-, second-, and third-generation EGFR-TKIs.

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2. Materials and methods

2.1. DNA extraction and sequencing

FFPE specimens were subjected to nucleic acid extraction. DNA was purified with the use of an Allprep DNA/RNA FFPE Kit (Qiagen, Valencia, CA) and were then subjected to NGS panels for mutation detection. For DNA sequencing, 10 ng of DNA were subjected to multiplex PCR amplification with the use of Ion AmpliSeq Colon and Lung Cancer Panel (Life Technologies), covering hot spots in 22 genes implicated in colon and lung cancers: *AKT1*, *ALK*, *BRAF*, *CTNNB1*, *DDR2*, *EGFR*, *ERBB2*, *ERBB4*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *KRAS*, *MAP2K1*, *MET*, *NOTCH1*, *NRAS*, *PIK3CA*, *PTEN*, *SMAD4*, *STK11*, and *TP53*. Sequencing was run on an Ion Torrent PGM instrument. Reads were aligned against the hg19 human reference genome, and variants were called with the use of Variant Call Format ver. 4.0. Germline mutations were excluded with the use of the Human Genetic Variation Database (http://www.genome.med.kyoto-u.ac.jp/SnpDB)

2.2. Cell culture and reagents

The Ba/F3 cell line was maintained in interleukin-3–supplemented RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum (Sigma-Aldrich). The cells were maintained under a humidified atmosphere of 5% CO_2 at 37 °C. The EGFR-TKIs gefitinib, erlotinib, afatinib, dacomitinib, neratinib, osimertinib, and rociletinib were obtained from Selleck Chemicals (Houston, TX).

2.3. Recombinant retrovirus production and cell infection

Recombinant retroviruses encoding the L858R or S768I mutant forms of human EGFR were generated and used to infect Ba/F3 cells as described previously [1]. Sequences of the polymerase chain reaction primers for mutagenesis are available on request. Both mutations were confirmed by sequencing analysis.

2.4. Cell growth inhibition assay

The growth-inhibitory effects of EGFR-TKIs on retrovirus-infected Ba/F3 cells were examined in medium without added interleukin-3 with the use of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously [1].

3. Results

3.1. Case report

A 74-year-old Japanese man with no smoking history was diagnosed with stage IIA (cT2aN1M0) lung adenocarcinoma in April 2011 and was scheduled for surgical resection (left pneumonectomy). However, pleural dissemination was detected during the operation, and he was referred to the Department of Medical Oncology at Kindai University Faculty of Medicine for further treatment of his advanced lung cancer. Salvage therapy consisting of five cycles of carboplatin, bevacizumab, and S-1 as the first line and of the combination of pemetrexed and a trial agent (antiangiogenic inhibitor) as the second line was administered. About 3 years after initiation of the second-line therapy, a positron emission tomography (PET)–computed tomography (CT) scan revealed a pathological fracture in lumbar vertebrae that was due to bone metastasis and was accompanied by a gradual increase in the serum concentration of carcinoembryonic antigen (CEA). Given his never-smoking history, a formalin-fixed paraffin-embedded tumor specimen obtained from the patient was subjected to comprehensive genomic profiling with clinical next-generation sequencing panels that cover mutational hotspots in 409 cancer-related genes [8]. The tumor was found to harbor two uncommon *EGFR* mutations—G719C in exon 18 and S768I in exon 20—as well as the E49K mutation of *KRAS* with mutation frequencies of 15.7%, 19.5%, and 6.6%, respectively (Fig. 1). On the basis of this finding, we treated the patient with afatinib at an oral dose of 40 mg daily as third-line therapy. After 1 month of afatinib treatment, his CEA level had decreased from 254.5 to 104.1 ng/ml (Fig. 2A). As a result of the development of skin toxicity of grade 2, the afatinib dose was reduced from 40 to 30 mg on day 36 and then to 20 mg on day 106, with an interruption in treatment from day 50 to day 63. A PET-CT scan revealed reduced [^{18}F]fluorodeoxyglucose uptake in his bone metastases at 11 months after initiation of afatinib treatment (Fig. 2B and C). To date, the patient has been receiving afatinib at a dose of 20 mg for >12 months and his CEA level remains below 5 ng/ml (Fig. 2A).

3.2. Sensitivity of Ba/F3 cells expressing EGFR(L858R) or EGFR(S768I) to EGFR-TKIs

There are still limited preclinical studies reporting the sensitivities of various kinds of EGFR-TKIs against uncommon EGFR mutations or KRAS mutations (Table 1). We therefore investigated whether the clinical efficacy of afatinib in the patient was reflected in the preclinical setting with Ba/F3 cells stably expressing EGFR(S768I). We examined the sensitivity of such cells as well as those expressing EGFR(L858R) to various EGFR-TKIs including first-generation (gefitinib, erlotinib), second-generation (afatinib, neratinib, dacomitinib), and third-generation (osimertinib,

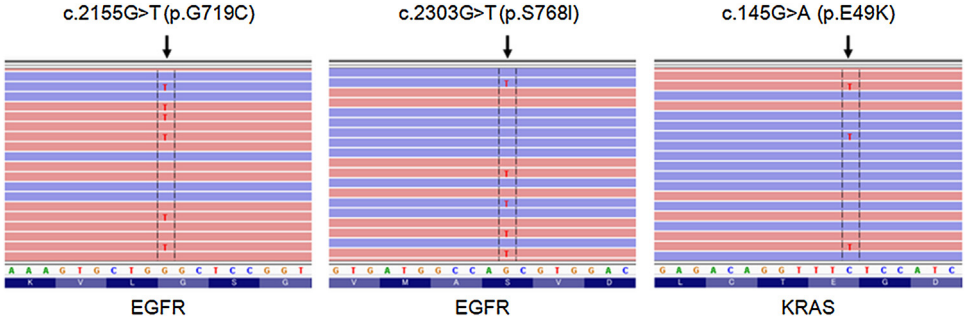

Fig. 1. Sequence traces for the G719C and S768I mutations of *EGFR* and the E49 K mutation of *KRAS* obtained for the NSCLC tumor of the patient.