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.

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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 dacotinib as well as the third-generation agents osimertinib, rocletinib, 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% CO2 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 IA (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–I as the first line and of the combination of pembrolizumab 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 [18F]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,
Table 1
IC_{50} values (nanomolar) of cells with various kinds of EGFR mutation or KRAS mutation.

<table>
<thead>
<tr>
<th>Mutations (Cell line)</th>
<th>IC_{50} (nM)</th>
<th>EGFR</th>
<th>Erlotinib</th>
<th>Gefitinib</th>
<th>Second generation</th>
<th>Afatinib</th>
<th>Netaritinib</th>
<th>Dacomitinib</th>
<th>Third generation</th>
<th>Osimertinib</th>
<th>Rociletinib</th>
<th>References</th>
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<tr>
<td>EGFR</td>
<td></td>
<td>ex18 del</td>
<td>882</td>
<td>884</td>
<td>1.7</td>
<td>27</td>
<td>29</td>
<td>93</td>
<td>999</td>
<td>[1]</td>
<td></td>
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<td></td>
<td></td>
<td>E709K</td>
<td>187</td>
<td>215</td>
<td>0.7</td>
<td>6</td>
<td>16</td>
<td>62</td>
<td>706</td>
<td>[1]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>G719A</td>
<td>213</td>
<td>167</td>
<td>0.9</td>
<td>1.1</td>
<td>6</td>
<td>53</td>
<td>214</td>
<td>[1]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>G719C</td>
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<td>300</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[16]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>G719S</td>
<td>500</td>
<td>300</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>[16]</td>
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<tr>
<td></td>
<td></td>
<td>ex19del</td>
<td>7</td>
<td>6</td>
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<td>27</td>
<td>1.6</td>
<td>1.1</td>
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<td></td>
<td></td>
<td>S768I</td>
<td>146</td>
<td>146</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td>49</td>
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<td></td>
<td></td>
<td>L858R</td>
<td>4.5</td>
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<td></td>
<td>L851Q</td>
<td>200</td>
<td>200</td>
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<td></td>
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<td>92</td>
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<td>KRAS</td>
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<td>G12A(H2009)</td>
<td>8800</td>
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<td>[17]</td>
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<td></td>
<td></td>
<td>G12C(HCC44)</td>
<td>7900</td>
<td></td>
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<td></td>
<td>[17]</td>
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<td></td>
<td></td>
<td>G12R(H157)</td>
<td>13800</td>
<td></td>
<td></td>
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<td></td>
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<td>[17]</td>
<td></td>
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<tr>
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<td></td>
<td>G125(A459)</td>
<td>9600</td>
<td>&gt;10000</td>
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<td></td>
<td></td>
<td>[17,18]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Q61H(H460)</td>
<td>12900</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>[17]</td>
<td></td>
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</table>

Note that biochemical IC_{50}s listed here were determined using different methods respectively and are therefore not directly comparable.

Table 2
IC_{50} values (nanomolar) of EGFR-TKIs for Ba/F3 cells stably expressing EGFR(L858R) or EGFR(S768I).

<table>
<thead>
<tr>
<th>EGFR-TKI</th>
<th>EGFR mutant</th>
<th>First generation</th>
<th>Second generation</th>
<th>Third generation</th>
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<tr>
<td></td>
<td>L858R</td>
<td>Gefitinib 6.0</td>
<td>Erlotinib 5.2</td>
<td>Osimertinib 2.5</td>
</tr>
<tr>
<td></td>
<td>S768I</td>
<td>Erlotinib 128</td>
<td>Afatinib 0.25</td>
<td>Rociletinib 16</td>
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<tr>
<td></td>
<td></td>
<td>Botacitinib 2.1</td>
<td>Neratinib 0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dacomitinib 2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Osimertinib 2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

rociletinib) agents. The median inhibitory concentration (IC_{50}) values of the drugs for cells expressing EGFR(S768I) were calculated (Table 2) and expressed as a ratio relative to those for cells expressing EGFR(L858R) (Fig. 3 and Supplemental Fig. 1). The IC_{50} ratios of both gefitinib and erlotinib were ~25, whereas those of osimertinib and rociletinib were 21.2 and 18.3, respectively. In contrast, the IC_{50} ratios for the second-generation drugs were much smaller, with afatinib showing the most similar growth-inhibitory effects in cells expressing the two different EGFR mutants (IC_{50} ratio of 3.3, 5.2, and 10.5 for afatinib, neratinib, and dacomitinib, respectively).

Fig. 2. Serum CEA levels and bone metastasis in the patient. (A) Time course of serum CEA concentration. Single solid arrow indicates the onset of afatinib treatment. Single and double dashed arrows indicate dose reductions of afatinib from 40 to 30 mg and from 30 to 20 mg, respectively. (B and C) PET-CT scans before and 11 months after the onset of afatinib treatment, respectively. The marked extent of [18F]fluorodeoxyglucose uptake apparent in lumbar vertebrae before afatinib treatment was greatly reduced after its onset.

Fig. 3. Relative growth-inhibitory effects of EGFR-TKIs in Ba/F3 cells stably expressing EGFR(S768I) or EGFR(L858R). Cells were cultured for 72 h in medium containing various concentrations of the indicated first-, second-, or third-generation (Gen) drugs, after which cell viability was assessed and the IC_{50} of each agent for cells expressing EGFR(S768I) relative to that for cells expressing EGFR(L858R) was calculated. Data are means of triplicates from a representative experiment.
These preclinical data are thus consistent with the observed clinical efficacy of afatinib in the patient.

4. Discussion

We have here shown the clinical efficacy of afatinib in a patient with NSCLC positive for G719C and S768I mutations of EGFR as well as for the E49K mutation of KRAS. This case has two unusual features: (1) Mutations of both EGFR and KRAS were present in the same patient, and (2) the tumor harbored two uncommon EGFR mutations.

Mutations of EGFR and KRAS rarely coexist in the same NSCLC tumor. Mutations in KRAS are generally considered to be a negative predictive factor for EGFR-targeted therapy [7], given that KRAS functions downstream of EGFR and that signaling by mutationally activated KRAS is therefore not inhibited by EGFR-targeted compounds. Compared with the most common KRAS mutations such as G12C/V/D, the E49K mutation, which was detected in the tumor of the present patient, has not been well characterized. The mutated residue is located in the switch III region of the protein, with mutations in this region having been shown to result in a moderate increase in RAS activity and tumorigenicity [9]. However, the clinical efficacy of afatinib in the present case suggests that activation of EGFR signaling by the G719C and S768I mutations, rather than signaling by KRAS(E49K), played the dominant role in carcinogenesis. Similarly, erlotinib was found to be effective in a NSCLC patient harboring both an exon 19 deletion of EGFR and a G13D mutation of KRAS [10], consistent with the notion that EGFR signaling might play the dominant role when mutant forms of both EGFR and KRAS coexist. On the other hand, the frequencies of the EGFR and KRAS mutations differed in the present patient, with values of 15.7% for EGFR(G719C), 19.5% for EGFR(S768I), and 6.6% for KRAS(E49K). It is therefore possible that activated KRAS signaling played a lesser role compared with activated EGFR signaling because fewer cells harbored the KRAS mutation.

Common EGFR mutations, which include exon 19 deletions and L858R in exon 21, confer sensitivity to EGFR-TKIs, whereas the effects of uncommon EGFR mutations remain largely unknown. Mutations in exon 18 have been detected in ~3% of EGFR mutation-positive NSCLC tumors, with G719X (where X indicates A, S, C, or D) accounting for most of these mutations [1]. The S768I mutation has previously been found to coexist with other EGFR mutations including G719X [11]. The sensitivity of these uncommon mutant forms of EGFR to EGFR-TKIs has been examined mostly with regard to first-generation agents, with gefitinib seeming to be clinically less active against EGFR(G719X) and EGFR(S768I) than against common mutant forms of the receptor [12,13]. We recently demonstrated the preclinical efficacy of afatinib in cells expressing EGFR(G719A) or EGFR(S768I) [1,14]. In the present study, we examined whether other second-generation EGFR-TKIs are also effective against EGFR(S768I). Afatinib exhibited the greatest preclinical efficacy among the second-generation EGFR-TKIs and was also markedly more effective than first- or third-generation agents, consistent with its observed clinical efficacy in the proband of this study. In spite of the limited number of patients (n = 8), combined data from a single-group phase II trial and randomized phase III trials of afatinib also reveal clinical efficacy of afatinib in individuals with NSCLC positive for both EGFR(G719X) and EGFR(S768I) [6], similar to the present case. A phase II trial of neratinib included 10 individuals with uncommon EGFR mutations among 102 EGFR mutation-positive NSCLC patients. Four patients harbored G719X mutations, three of whom achieved a partial response to neratinib treatment [15], suggestive of efficacy of this agent against tumors bearing G719X mutations. Together, the present and previous studies thus suggest that patients with NSCLC positive for uncommon EGFR mutations might derive benefit from treatment with afatinib or, possibly, with other second-generation EGFR-TKIs, but not from that with first- or third-generation agents. Given the fact that the tumor specimen was obtained at the time of diagnosis, there would be the possibility that mutational status had been changed after the exposure to chemotherapies. Further studies are necessary to substantiate this conclusion.

The development and implementation of comprehensive genomic profiling tests should offer more opportunities to identify tumors that harbor uncommon mutations of EGFR with or without coexisting mutations of other oncogenes. Given that oncogenicity and affinity for EGFR-TKIs differ among mutant forms of EGFR, it will be important to select the most appropriate agent for therapy in each case. It will also be of importance to determine the effectiveness of newly developed EGFR-TKIs against tumors harboring uncommon mutations of EGFR.

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Conflict of interest

Y.T. has received a lecture fee from Boehringer-Ingelheim Japan K.K. H.H. has received lecture fees from AstraZeneca K.K., Bristol-Myers Squibb, Chugai Pharmaceutical Co. Ltd., Eli Lilly Japan K.K., Ono Pharmaceutical Co. Ltd., and Taiho Pharmaceutical Co. Ltd. as well as advisory fees from AstraZeneca K.K., Boehringer-Ingelheim Japan Inc., and Eli Lilly Japan K.K. H.K received a lecture fee from Boehringer-Ingelheim Japan Inc. K. Nishio has received lecture fees from Daiichi Sankyo Co. Ltd., and Chugai Pharmaceutical Co. Ltd. K. Nakagawa has received lecture fees from Astra-Zeneca K.K., Boehringer-Ingelheim Japan Inc., Chugai Pharmaceutical Co. Ltd., Clovis, and Pfizer. All other authors declare no potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jlungen.2016.09.001.

References