A long-term responding epidermal growth factor receptor mutated non-small cell lung cancer patient with extremely high mutation allele frequency

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Epidermal growth factor receptor (EGFR) mutation is the most frequent oncogenic driver in non-small cell lung cancer (NSCLC) [1, 2]. Among EGFR mutations, exon 19 deletion and exon 21 L858R are two of the most common mutations; hence they are referred to as common mutations [1]. In addition to them, there are many types of EGFR mutation other than common mutations. They are treated as uncommon mutations [3, 4]. Recent advances in analysis technology using next-generation sequencing (NGS) have made great progress also in the area of EGFR mutation [5–8]. By this analytic method, it has become clear that there is heterogeneity among patients with common mutations. Among these possible heterogeneities, mutation allele frequency (MAF) has drawn attention [9–13]. MAF is defined as the number of times a mutated base is observed, divided by the total number of times any base is observed at the locus. It corresponds to the percentage of sequencing reads that contain the mutation, and the proportion of alleles is affected by the proportion of tumor cells in the sample and the presence of copy number alterations [9–13]. Here we report a case of a patient with EGFR mutation with very high MAF, who responded to EGFR-tyrosine kinases for a long time.

An 82-year-old woman was referred to our hospital for the treatment of EGFR mutated adenocarcinoma of the lung. Her performance status (ECOG) was 0, and clinical stage was stage IVA. She was diagnosed as having EGFR mutated (exon 21 L858R) adenocarcinoma of the lung. Gefitinib was effective for 28 months. Since the adenocarcinoma recurred, afatinib was administered and it responded for 24 months. Due to the gradual progression of dementia during this course of treatment, afatinib treatment was terminated.

The content ratio of tumor cells in the tissue sample and MAF were examined using NOIR-SS (DNA Chip Research Inc. Tokyo, Japan) [6]. In brief, DNA was extracted from slices of FFPE tissue block of the patient using a Maxwell RSC DNA FFPE kit (Promega, Madison, USA). 50 ng of DNAs were fragmented by a Covaris Focused-ultrasonicator (Woburn, MA, USA) and a molecular barcoded next generation sequencing library was constructed by the NOIR-SS method as described previously [6]. The constructed library was sequenced using the Ion Chef/Ion S5 platform with Ion 540 chip (Thermo Fisher Scientific, Waltham, MA, USA). The patient's L858R MAF was 90.1%, the highest ever measured. This result suggested the presence of homozygotes or EGFR copy gain.

The most frequent driver genes in NSCLC are EGFR mutations. Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) prolonged the survival of patients with this mutation. However, the response and duration of the response are not uniform among patients, and further understanding of EGFR is required. Recent advances in NGS have dramatically increased information about EGFR mutations. Among them, MAF is attracting attention [9–13]. MAF corresponds to the percentage of sequencing reads that contain the mutation, and the proportion of alleles is affected by the proportion of tumor cells in the sample and the presence of copy number alterations [6]. However, the proportion of cells within the tumor that carry the mutation is evaluated as most important. Mutations that occur early in tumor evolution have a higher allelic frequency than late, subclonal mutations. They are drivers of cancer evolution and can be attractive therapeutic targets. Most EGFR mutations are clonal. Although it is difficult to calculate an accurate estimate of the clone ratio, allele frequency is readily available and can be a useful surrogate. In a Shizuoka Lung Cancer Mutation Study of 102 lung adenocarcinoma patients with the typical EGFR exon 21 L858R, the median MAF of L858R was 18.5% (8–82%) [10]. In their analysis using receiver operating characteristic curves, a MAF of 9% resulted in 100% sensitivity and 99% specificity [10]. Using this cutoff value, they investigated the effect of allelic frequency on survival in patients treated with TKIs. The result is that the progression-free survival among patients with a MAF ≤ 9% was 92 days, compared to 284 days for those with a frequency greater than 9% [10]. Li *et al.* reported that the median MAF was 25.8%, with a range of 1.4–86.2% in 194 NSCLC patients with exon 19 deletion and exon 21 L858R of the largest available dataset

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based on a prospective study from the phase III CTONG 0901 trial [11]. Recently, Friedlaender *et al*. reported that the median allelic frequency of the EGFR mutation was 47% (interquartile range: 24–65%) in 31 NSCLC patients with exon 19 deletion and exon 21 L858R treated with first-line TKIs [12]. Although the driver gene was different from EGFR mutation, an association between high MAF level and good response was reported in a lung cancer patient with MET exon 14 skipping mutation. In this patient, MAF was 73.9% [13].

Our patient had exon 21 L858R, and MAF of EGFR L858R was very high at 90.1%. To the best of our knowledge, no report showing such a high value of MAF has been published. This patient received long-term first-line therapy with gefitinib followed by second-line therapy with afatinib, which also had a long-term response. It was suggested that such high MAF might have been the result of homozygotes or EGFR copy gain. In other words, it was considered that NSCLC in this patient was highly dependent on EGFR, and as a result, a long duration of response was obtained. This patient was aged and did not wish to have an invasive examination, so the pathological samples could not be obtained at the time of recurrence. It was interesting to evaluate what the MAF was at the time of recurrence from the viewpoint of overcoming the resistance of EGFR-TKIs. If it becomes possible to evaluate with a sample obtained by a non-invasive method such as serum, TKI treatment might be further dramatically improved.

Our patient had a very high MAF level of EGFR L858R and responded well in the long term. Information about MAF in EGFR mutation might provide useful regarding the treatment of patients with this mutation.

The authors declare no conflict of interest.

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