Frequency of EGFR T790M mutation and multimutational profiles of rebiopsy samples from non-small cell lung cancer developing acquired resistance to EGFR tyrosine kinase inhibitors in Japanese patients

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Abstract

Background: The majority of non-small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) mutation eventually develop resistance to EGFR tyrosine kinase inhibitors (TKIs). Minimal information exists regarding genetic alterations in rebiopsy samples from Asian NSCLC patients who develop acquired resistance to EGFR-TKIs.

Methods: We retrospectively reviewed the medical records of patients with NSCLC harboring EGFR mutations who had undergone rebiopsies after developing acquired resistance to EGFR-TKIs. We analyzed 27 practicable samples using a tumor genotyping panel to assess 23 hot-spot sites of genetic alterations in nine genes (EGFR, KRAS, BRAF, PIK3CA, NRAS, MEK1, AKT1, PTEN, and HER2), gene copy number of EGFR, MET, PIK3CA, FGFR1, and FGFR2, and ALK, ROS1, and RET fusions. Additionally, 34 samples were analyzed by commercially available EGFR mutation tests.

Results: Sixty-one patients underwent rebiopsy. Twenty-seven samples were analyzed using our tumor genotyping panel, and 34 samples were analyzed for EGFR mutations only by commercial clinical laboratories. Twenty-one patients (34%) had EGFR T790M mutation. Using our tumor genotyping panel, MET gene copy number gain was observed in two of 27 (7%) samples. Twenty patients received continuous treatment with EGFR-TKIs even after disease progression, and 11 of these patients had T790M mutation in rebiopsy samples. In contrast, only 10 of 41 patients who finished EGFR-TKI treatment at disease progression had T790M mutation. The frequency of T790M mutation in patients who received continuous treatment with EGFR-TKIs after disease progression was significantly higher than that in patients who finished EGFR-TKI treatment at disease progression (55% versus 24%, p = 0.018).

Conclusions: The frequency of T790M mutation in this study was lower than that in previous reports examining western patients. These results suggest that continuous treatment with EGFR-TKI after disease progression may enhance the frequency of EGFR T790M mutation in rebiopsy samples.

Keywords: Non-small cell lung cancer, Epidermal growth factor receptor mutation, Rebiopsy, T790M mutation

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Background
Lung cancer is the most common cause of cancer-related deaths, and non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers [1, 2]. Over 70% of patients with NSCLC have advanced disease at the time of diagnosis, and prognosis is generally poor [3]. Recently, molecular targeted therapies have been developed and have provided a remarkable benefit to NSCLC patients with specific genetic alterations. In particular, NSCLC with mutation in the epidermal growth factor receptor (EGFR) gene are sensitive to EGFR blockade with specific tyrosine kinase inhibitors (TKIs). EGFR-TKIs are efficacious in patients with NSCLC harboring EGFR mutations as demonstrated in prospective clinical trials [4–8]. However, in spite of this efficacy almost all patients with EGFR-mutant NSCLC develop resistance to EGFR-TKIs.

Various mechanisms of resistance to EGFR-TKIs have been identified, and understanding these is critical for development of effective treatment strategies for EGFR-TKI-resistant NSCLC. The major mechanism of acquired resistance reported is secondary T790M mutation on exon 20 on the EGFR gene [9–12]. This secondary mutation enhances ATP-binding affinity of EGFR-mutated cells. Since EGFR-TKIs are competitive ATP-inhibitors, their efficacy is decreased in the face of the T790M mutation [13]. Additional mechanisms include amplification of the MET gene [11, 12, 14], PIK3CA mutation [11, 15], BRAF mutation [16], epithelial-mesenchymal transition (EMT) [11], and small cell lung cancer (SCLC) transformation [11, 12].

Several studies have examined the mechanisms and frequency of EGFR-TKI resistance, though minimal data regarding Japanese patients exist. Furthermore, the clinical factors that influence the frequency of acquired resistance mutations, especially T790M, remain unclear. This study aimed to analyze the causes of acquired resistance to EGFR-TKIs in Japanese patients with NSCLC, and to evaluate clinical factors related the frequency of T790M mutation.

Methods

Patients
We reviewed the medical records of consecutive patients with NSCLC harboring EGFR mutations who had undergone rebiopsies based on physician’s decision in the cases of acquired resistance to EGFR-TKI. Most rebiopsy samples were obtained from sites assessed as disease progression by imaging. Patients were treated at the Shizuoka Cancer Center between September 2002 and August 2014. Acquired resistance was defined according to Jackman’s criteria [17]. The criteria defined acquired resistance as progression while receiving EGFR-TKI, after initial response or durable stable disease (>6 months). The written informed consent regarding EGFR mutational analysis was obtained from most patients, and verbal informed was from some patients since EGFR mutational analysis was performed under the Japanese insurance system. Additionally, some patients were enrolled in the Shizuoka Lung Cancer Mutation Study [18], and these samples were analyzed using our tumor genotyping panel. This study protocol was approved by the Institutional Review Board of Shizuoka Cancer Center under number 27–J102–27–1–3.

Mutational profiling
A tumor genotyping panel was designed to assess 23 hotspot sites of genetic alterations in 9 genes (EGFR, KRAS, BRAF, PIK3CA, NRAS, MEK1, AKT1, PTEN, and HER2), gene copy number of EGFR, MET, PIK3CA, FGFR1, and FGFR2, and ALK, ROS1, and RET fusions using pyrosequencing plus capillary electrophoresis, quantitative polymerase chain reaction (PCR), and reverse transcription PCR, respectively (Table 1). We analyzed samples from patients enrolled in the Shizuoka Lung Cancer Mutation Study, using this tumor genotyping panel. The other samples were analyzed for EGFR mutations using the Scorpion ARMS or Cycleave methods by a commercial clinical laboratory (SRL Inc., Tokyo, Japan) (see Additional file 1).

Evaluation of efficacy
Responsiveness to EGFR-TKI treatment was evaluated according to the Response Evaluation Criteria in Solid Tumors version 1.1 [19]. Progression-free survival (PFS) was defined as the period between the start of EGFR-TKI treatment and progressive disease or death from any cause. Overall survival (OS) was defined as the period between the start of EGFR-TKI treatment and the date of death from any cause.

Statistical analysis
All categorical variables were analyzed by the chi-square test or Fisher’s exact test, as appropriate. Continuous variables were analyzed using the Mann-Whitney test. Logistic regression analyses were used to adjust for potential confounding factors. All p values < 0.05 were considered statistically significant. All analyses were performed using JMP 10 for Windows statistical software (SAS Institute Japan Inc., Tokyo, Japan).

Results

Patient characteristics
Sixty-one patients with NSCLC harboring EGFR mutations, and who had undergone rebiopsy after acquired resistance to EGFR-TKI at the Shizuoka Cancer Center were included in this study. Patient characteristics are shown in Table 2. The median age (range) was 64 (39–84) years, and most patients were female (72%) and never-smokers.
All patients had been diagnosed with adenocarcinoma of the lung with activating $EGFR$ mutations at initial diagnosis. The types of $EGFR$ mutations before the initial $EGFR$-TKI treatment were exon 19 deletion in 37 patients (61 %), exon 21 L858R in 19 patients (31 %), and other/double $EGFR$ mutations in five patients (8 %). Thirty-nine patients (64 %) were treated with $EGFR$-TKI as first-line therapy. Twenty-two patients (36 %) received $EGFR$-TKI as second or subsequent-line therapy. Forty-nine patients (80 %) were treated with gefitinib, seven patients (12 %) with erlotinib, and five patients (8 %) with other $EGFR$-TKIs including afatinib. All patients received $EGFR$-TKI monotherapy. Twenty patients received continuous treatment with $EGFR$-TKI more than 30 days after disease progression, and 41 patients finished $EGFR$-TKI treatment within 29 days after diagnosis of disease progression.

**Table 1** Multiplexed tumor genotyping panel

| Gene name | Position | AA mutant | Nucleotide mutant |
|-----------|----------|-----------|-------------------|
| $EGFR$    | G719     | G719      | 2155G > T/A       |
|           | G719A    | G719      | 2156G > C         |
|           | exon 19  | Deletion  |                   |
|           | T790     | T790M     | 2369C > T         |
|           | exon 20  | Insertion |                   |
| L858      | L858R    | 2573 T > G|
| L861      | L861Q    | 2582 T > A|
| $KRAS$    | G12      | G12C/S/R  | 34G > T/A/C       |
|           | G12V/A/D | 35G > T/C/A|
|           | G13      | G13C/S/R  | 37G > T/A/C       |
|           | G13D/A   | 38G > A/C |
|           | Q61      | Q61K      | 181C > A          |
|           | Q61/L    | 182A > G/T|
|           | Q61H     | 183A > T/C|
| $BRAF$    | G466     | G466V     | 1397G > T         |
|           | G469     | G469A     | 1406G > C         |
|           | L597     | L597V     | 1789C > G         |
|           | V600     | V600E     | 1799 T > A        |
| $PIK3CA$  | E542     | E542K     | 1624G > A         |
|           | E545     | E545K/Q   | 1633G > A/C       |
|           | H1047    | H1047R    | 3140A > G         |
| $NRAS$    | Q61      | Q61K      | 181C > A          |
|           | Q61/L/R  | 182A > T/G|
| $MEK1$ (MAP2K1) | Q56 | Q56F | 167A > C |
|           | K57      | K57N      | 171G > T         |
|           | D67      | D67N      | 199G > A         |
| $AKT1$    | E17      | E17K      | 49G > A          |
| $PTEN$    | R233     | R233      | 697C > T         |
| $HER2$    | exon 20  | Insertion |                   |

**Table 2** Patient characteristics analyzed in our study ($n = 61$)

| Characteristic | Value |
|---------------|-------|
| Age, year     | 64    |
| Sex, n (%)    |       |
| Female        | 44 (72 %) |
| Male          | 17 (28 %) |
| Smoking history, n (%) |       |
| Never         | 44 (72 %) |
| Former/Current| 17 (28 %) |
| ECOG performance status, n (%) |       |
| 0–1           | 52 (85 %) |
| 2–4           | 9 (15 %) |
| Pretreatment $EGFR$ status, n (%) |       |
| Exon19 deletion | 37 (61 %) |
| Exon21 L858R  | 19 (31 %) |
| Other         | 5 (8 %) |

| $EGFR$ TKI, n (%) |       |
| Gefitinib        | 49 (80 %) |
| Erlotinib        | 7 (12 %) |
| 2nd generation   | 5 (8 %) |

Abbreviations: ECOG eastern cooperative oncology group, EGFR epidermal growth factor receptor, TKI tyrosine kinase inhibitor

**Rebiopsy**

Table 3 depicts characteristics of rebiopsy sites, specimens, and procedures in patients who had undergone rebiopsy after developing acquired resistance to $EGFR$-TKIs. Because of their easy accessibility and practical necessity, serous effusions such as pleural effusion and cerebrospinal fluid account for more than half of the specimens. Pulmonary lesions were also rebiopsied, with the most common procedure being transbronchial biopsy. Biopsy samples from lymph nodes or other sites were obtained using computed tomography-guided or sonography-guided needle biopsy. All rebiopsies were performed after stopping $EGFR$-TKI treatment.

**Resistance mechanisms**

A total of 61 rebiopsy samples were analyzed for $EGFR$ mutations. Twenty-seven rebiopsy samples were analyzed using our tumor genotyping panel, and 34 samples were examined for $EGFR$ mutations by commercial clinical laboratories. All of 61 patients had $EGFR$ activating mutations before $EGFR$-TKI treatment, and 55 patients (90.2 %) still had same $EGFR$ mutations in rebiopsy samples. T790M mutation was identified in 21 of 61 samples (34.4 %; Fig. 1). No samples had small cell histologic transformation. In samples analyzed using our tumor genotyping panel, $MET$ gene copy number gain was seen in two of 27 samples (7 %). Additionally, we detected...
PIK3CA mutation (E542K), BRAF mutation (G466V), and KRAS mutation (G12D), in one sample each in 27 samples (4%) (Fig. 2). Six of 61 rebiopsy samples (9.8%) did not possess EGFR mutation, despite having EGFR activating mutations at the initial analysis. KRAS mutation was detected in 1 of these samples.

T790M prevalence
Correlations between patient characteristics and T790M prevalence were evaluated (Table 4). Eleven of 20 patients who received continuous treatment with EGFR-TKI after disease progression had T790M mutation in the rebiopsy sample. However, only 10 of 41 patients who had finished EGFR-TKI treatment at the time of disease progression had T790M mutation (Fig. 3). The frequency of T790M mutation in patients who received continued treatment with EGFR-TKI after disease progression was significantly higher than in patients who finished EGFR-TKI at diagnosis of disease progression (55% versus 24%, p = 0.018). Multivariate analysis also demonstrated that continuous treatment with EGFR-TKI after disease progression was significantly correlated with T790M mutation (Table 4). Other characteristics, including PFS with EGFR-TKI, rebiopsy site, and rebiopsy sample, had no statistical association with the prevalence of T790M.

Discussion
Previous reports from examining patients in western countries have reported EGFR T790M mutation in 49–69% patients with NSCLC harboring EGFR mutations who had undergone rebiopsy after developing acquired resistance to EGFR-TKIs [11, 12, 20]. In contrast, our study identified T790M mutation in only 21 of 61 rebiopsy samples (34.4%). This finding is similar to that of the one other Japanese study we are aware of [21]. Therefore, T790M prevalence in Japanese and Western patients may be different. In our study, only 30% of patients received continuous treatment with EGFR-TKI after disease progression. Similarly, few such patients were included in the study from Hata et al. [21]. However, 88–91% of patients in previous studies from western countries received continuous treatment with EGFR-TKI after disease progression [12, 20]. Additionally, the frequency of T790M mutation in patients who received continuous treatment with EGFR-TKI after disease progression was significantly higher than that in patients who had finished EGFR-TKI treatment by diagnosis of disease progression in our study. Furthermore, the preclinical report showed that continuous exposure to EGFR-TKIs induced T790M mutation in a NSCLC cell line with an EGFR-sensitive mutation [22]. These data suggest that continued treatment with EGFR-TKIs after disease progression may promote T790M mutation. While differences in ethnicity and analysis methods may underlie these inconsistencies, the potential for EGFR-TKIs to promote T790M mutation should not be overlooked.

Table 3

| Procedure and specimen | Number |
|------------------------|--------|
| Surgery                |        |
| Brain                  | 3      |
| Lung                   | 2      |
| Autopsy                | 1      |
| Biopsy                 |        |
| Lung                   | 15     |
| Lymph node             | 3      |
| Other                  | 4      |
| Fluid                  |        |
| Pleural effusion       | 24     |
| Cerebrospinal fluid    | 8      |
| Cardiac effusion       | 1      |

Fig. 1 Frequency of T790M mutation in rebiopsy samples (n = 61)

Fig. 2 Multimutational profiling in rebiopsy samples analyzed using our tumor genotyping panel (n = 27). CNG: Copy number gain
The frequencies of MET gene copy number gain and PIK3CA mutation in our study were similar to those previously reported in studies from western countries [11, 12]. Furthermore, BRAF mutation is associated with acquired resistance to EGFR-TKIs [16]. We also detected KRAS mutation in one rebiopsy sample. KRAS and EGFR mutations have previously been considered mutually exclusive [23]. However, Kuiper et al. recently reported KRAS mutation in one rebiopsy sample following development of acquired resistance to EGFR-TKIs [24].

| Patient characteristics | Number | T790M (%) | P (Univariate) | P (Multivariate) |
|-------------------------|--------|-----------|----------------|------------------|
| Age                     |        |           |                |                  |
| ≥75                     | 12     | 4 (33 %)  |                |                  |
| <74                     | 49     | 17 (35 %) |                |                  |
| Sex                     |        |           |                |                  |
| Female                  | 44     | 14 (32 %) |                |                  |
| Male                    | 17     | 7 (41 %)  |                |                  |
| Smoking history          |        |           |                |                  |
| Never                   | 44     | 14 (32 %) |                |                  |
| Former/current          | 17     | 7 (41 %)  |                |                  |
| EGFR mutation status    |        |           |                |                  |
| Exon19 deletion         | 37     | 9 (24 %)  |                |                  |
| Exon21 L858R            | 19     | 9 (47 %)  |                |                  |
| Other                   | 5      | 3 (60 %)  |                |                  |
| Rebiopsy site           |        |           |                |                  |
| Central nervous system  | 11     | 3 (27 %)  |                |                  |
| Other                   | 50     | 18 (36 %) |                |                  |
| Rebiopsy sample         |        |           |                |                  |
| Tissue                  | 28     | 12 (43 %) |                |                  |
| Fluid                   | 33     | 9 (27 %)  |                |                  |
| EGFR TKI                |        |           |                |                  |
| Gefitinib               | 49     | 17 (35 %) |                |                  |
| Erlotinib               | 7      | 4 (57 %)  |                |                  |
| 2nd generation          | 5      | 0 (0 %)   |                |                  |
| Line of EGFR-TKI        |        |           |                |                  |
| 1st                     | 39     | 12 (31 %) |                |                  |
| 2nd or later            | 22     | 9 (41 %)  |                |                  |
| History of platinum doublet until rebiopsy | |        |                |                  |
| Yes                     | 34     | 11 (32 %) |                |                  |
| No                      | 27     | 10 (37 %) |                |                  |
| PFS with EGFR-TKI       |        |           |                |                  |
| ≥10 months              | 34     | 13 (38 %) |                |                  |
| <10 months              | 27     | 8 (30 %)  |                |                  |
| Interval between RECIST PD and rebiopsy | |        |                |                  |
| ≥4 months               | 29     | 12 (41 %) |                |                  |
| <4 months               | 32     | 9 (28 %)  |                |                  |
| Period of continuation of TKI beyond PD | |        |                |                  |
| ≥30 days                | 20     | 11 (55 %) |                |                  |
| <30 days                | 41     | 10 (24 %) |                |                  |

Abbreviations: EGFR epidermal growth factor receptor, TKI tyrosine kinase inhibitor, PFS progression free survival, PD progressive disease
Furthermore, Li et al. have identified double mutation of EGFR and KRAS in pretreatment assessment of NSCLC patients [25]. These data suggest that KRAS mutation may promote acquired resistance to EGFR-TKIs through drug selective pressure. However, more data are required to confirm this hypothesis.

The availability of continuous treatment with EGFR-TKIs after disease progression is still controversial. In IMPRESS trial, continuation of gefitinib treatment after disease progression on gefitinib monotherapy did not prolong progression-free survival and overall survival in patients who received platinum-based doublet chemotherapy as subsequent line of treatment [26]. However, it is unclear that the efficacy of continuous using EGFR-TKIs without platinum doublets [27, 28]. Recently, we had been able to use third generation EGFR-TKIs that have great efficacy for NSCLC with EGFR T790M mutation in clinical practice. If there are relationship between the continuous treatment with EGFR-TKIs after disease progression and the frequency of T790M, the continuous therapy can be more important choice.

Our study had several limitations. First, we retrospectively collected the data from a single institution, and our sample size was small. This small sample size results from the difficulty surrounding rebiopsy in clinical practice. Second, we analyzed only 27 rebiopsy samples (44.3 %) using our tumor genotyping panel. Therefore, further multi-institutional studies are warranted to verify our results.

**Conclusions**

The frequency of T790M mutation in rebiopsy samples in our study was lower than that reported in previous reports studies of western patients. The frequency of T790M mutation in patients who received continuous treatment with EGFR-TKIs after disease progression was significantly higher than that in patients who stopped EGFR-TKI treatment at diagnosis of disease progression. Continuous treatment with EGFR-TKI following disease progression may therefore influence the frequency of EGFR T790M mutations in rebiopsy samples.

**Additional file**

**Additional file 1:** The detail of mutational analysis. (DOCX 22 kb)

**Abbreviations**

EGFR: Epidermal growth factor receptor; EMT: Epithelial-to-mesenchymal transition; NSCLC: Non-small cell lung cancer; OS: Overall survival; PCR: Polymerase chain reaction; PFS: Progression-free survival; SCLC: Small cell lung cancer; TKI: Tyrosine kinase inhibitor

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Availability of data and materials
The datasets supporting the conclusions of this article are included within the manuscript and Additional file 1.

Authors’ contributions
RK contributed to the drafting of this manuscript and data collection, and HK contributed to the study design and statistical analysis. MS, YK contributed to analysis of the samples using our tumor genotyping panel. KW, AO, TT, TN, HM, MI, ME, TN, YO, NY, KT, and TT contributed to analysis of the data and interpretation of the findings. All authors have read and approved the submission of the final manuscript.

Competing interest
The authors declare that they have no competing interest.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study protocol was approved by the Institutional Review Board of Shizuoka Cancer Center Research Institute, 1007 Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan.

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