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Diagnostic significance of cerebrospinal fluid *EGFR* mutation analysis for leptomeningeal metastasis in non-small-cell lung cancer patients harboring an active *EGFR* mutation following gefitinib therapy failure

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ABSTRACT

Background: Epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors (TKIs) have been successfully used to treat patients with non-small cell lung cancer (NSCLC) harboring *EGFR* mutations. However, despite an initial excellent response, recurrence within one or two years is common. Diagnosis and treatment of leptomeningeal metastasis (LM), a form of NSCLC recurrence, remains particularly difficult. Here, we analyzed the *EGFR* mutation status of cerebrospinal fluid (CSF) directly using real-time polymerase chain reaction (PCR) and evaluated the efficacy of therapy with erlotinib, an *EGFR* TKI.

Patients and methods: Seven NSCLC patients harboring activating *EGFR* mutations who had developed LM during or after therapy with gefitinib, an *EGFR* TKI, were retrospectively analyzed. CSF was obtained and subjected to cytological examination and *EGFR* mutation analysis, including detection of the resistance-associated T790M mutation, using real-time PCR.

Results: In all seven cases, the *EGFR* mutation detected in the CSF was the same as that detected in the primary tumor (sensitivity, 100%). Conversely, cytology results were positive in only two patients (sensitivity, 28.6%). No additional T790M mutations were detected. Erlotinib was efficacious in all cases, and improved performance status was achieved for five of the seven patients. The effect of erlotinib treatment was temporary, however, with time to treatment failure (TTF) ranging from 29 to 278 days (median, 65 days) and the interval between commencement of erlotinib treatment and death ranging from 45 to 347 days (median, 168 days).

Conclusions: Analysis of *EGFR* mutations in CSF using a highly sensitive real-time PCR assay is a potentially powerful diagnostic method for LM.

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1. Introduction

In East Asia, and especially in Japan, almost half of all patients with lung adenocarcinoma may have tumors that harbor epidermal growth factor receptor (*EGFR*) mutations. Tyrosine kinase inhibitors (TKIs) such as gefitinib have been widely used to treat these patients, often with excellent therapeutic results. However, despite initial remission, recurrence within one or two years (median, 10 months) is common [1]. Relapse often takes the form of leptomeningeal metastasis (LM), and a recent study found that up to 60% of patients with advanced non-small cell lung cancer (NSCLC) will develop central nervous system (CNS) metastases, including LM, during the course of gefitinib therapy [2]. The overall incidence of LM is reported to be 10–26% in lung cancer patients [3], although this may be an underestimate. The sensitivity of cerebrospinal fluid (CSF) cytology for detecting metastasis is reported to be only approximately 50% in patients with LM [4]. Furthermore, despite the development of new diagnostic tools such as CSF flow cytometry and the identification of various new biomarkers, diagnostic accuracy remains unsatisfactory [5].

A number of treatment strategies have been tried for patients with *EGFR* mutation-harboring NSCLC that has metastasized to the CNS after gefitinib therapy, including the administration of high-dose gefitinib [6] and alternating the administration of erlotinib, another *EGFR* TKI [7]. We designed this study to establish the diagnostic potential of *EGFR* mutations, including the resistance-associated T790M mutation, in CSF from adenocarcinoma patients harboring activating *EGFR* mutations who have LM after gefitinib therapy. Moreover, we administered erlotinib to patients without the T790M mutation in the CSF and evaluated its efficacy.

2. Patients and methods

We retrospectively analyzed NSCLC patients harboring activating *EGFR* mutations who had developed LM during or after gefitinib therapy at our institution between February 2010 and June 2011. With written informed consent, CSF was obtained and subjected to cytological examination and *EGFR* mutation analysis, including deletion of exon 19 and point mutation of L858R and T790M, using a cycleave real-time polymerase chain reaction (PCR) assay as previously described [8,9]. Briefly, genomic DNA was extracted, and exons 19, 20, and 21 of the *EGFR* gene were amplified using real-time quantitative PCR on a SmartCycler (TaKaRa, Gifu, Japan) with Cycleave PCR™ Core Kits (TaKaRa) with specific cycling probes, including a deletion of exon 19, a L858R, a T790M, and a wild-type. The assays were conducted by Special Reference Laboratories, Inc. (Tokyo, Japan).

After determining the status of the *EGFR* gene in the CSF, we administered erlotinib to the patients who did not have the resistance-associated T790M mutation and evaluated its efficacy in terms of amelioration of neurological symptoms and improvements in performance status. We also measured time to treatment failure (TTF). This study was approved by the ethics committee of Juntendo University Urayasu Hospital (No. Juntendo-Urayasu-Rinri 26-7). Patients' data were used following the provision of comprehensive consent by the patients.

3. Results

The characteristics and clinical courses of the seven patients who met the study criteria (6 women, 1 man) are summarized in Tables 1 and 2. Patient ages ranged from 54 to 71 years (median, 58 years). All patients had a histological tumor classification of adenocarcinoma. The type of *EGFR* mutation in the primary tumor was deletion of exon 19 (X19del) in four patients and point mutation in exon 21 (L858R) in the other three patients. In all seven patients, the *EGFR* mutation detected in the CSF was the same as that detected in the primary tumor (sensitivity, 100%; Tables 1 and 2), whereas cytology results were positive in only two patients (sensitivity, 28.6%; Table 2). No additional mutations, such as T790M mutation, were detected (Table 2).

Amelioration of neurological symptoms was observed in all patients who received erlotinib therapy. Furthermore, an improvement in performance status was achieved in five of these patients. However, remission after erlotinib treatment was temporary, as expected, with the TTF ranging from 29 to 278 days (median, 65 days) (Fig. 1A) and the interval between commencement of erlotinib treatment and death ranging from 45 to 347 days (median, 168 days) (Fig. 1B). The response to erlotinib in the extracranial sites was basically poor, with gradual progression in these sites despite erlotinib administration in all patients but one. In case 5, efficacy was demonstrated as a stable disease for approximately three months. The time course for each patient is shown in Fig. 2, and the findings for the male patient (No. 5), for whom both pleural effusion and CSF as a second biopsy specimen were available, are shown in supplemental Fig. S1.

The adverse events associated with erlotinib, such as skin rash, diarrhea, and liver dysfunction, were limited and tolerable (grade 1–2). The median overall survival was 776 days (range, 374–1269 days).

4. Discussion

In this study, analysis of the *EGFR* mutation status in the CSF was more sensitive than cytology to diagnose LM in patients with a primary tumor carrying an *EGFR* mutation (sensitivity of

Abbreviations: *EGFR*, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer; LM, leptomeningeal metastasis; CSF, cerebrospinal fluid; PCR, polymerase chain reaction; TTF, time to treatment failure; CNS, central nervous system; *HER2*, human epidermal growth factor receptor 2; WBRT, whole brain radiotherapy

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