Downregulation of EGFR in a metastatic brain lesion of EGFR-mutated non-small cell lung cancer using a tyrosine kinase inhibitor: A case report

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Abstract. Brain metastasis is a common complication in patients with cancer, with lung cancer being the most frequent origin of brain metastases. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have begun to serve a pivotal role in lung cancer treatment and have been reported to demonstrate anticancer activity against brain metastases by penetrating the blood-brain barrier. The present study reports, to the best of our knowledge, the first case of EGFR-mutated non-small cell lung cancer (NSCLC) brain metastasis that was surgically resected while the lesion was responding to the EGFR-TKI erlotinib. The results of the present study demonstrated that EGFR-mutated NSCLC cells were able to evade the cytotoxic effect of EGFR-TKI by downregulating EGFR expression, without exhibiting the T790M EGFR mutation.

Introduction

Brain metastasis is a common complication in patients with cancer, with lung cancer being the most frequent origin of brain metastases (1). Lung cancer has an ~40% chance of metastasizing to the brain during the course of disease progression (1). Current standard treatments for lung cancer brain metastasis patients include surgery, stereotactic radiosurgery and whole-brain radiation therapy (1). It has long been considered that the blood-brain barrier (BBB) hampers the delivery of chemotherapeutic agents to the metastatic brain lesion, and systemic antineoplastic therapy has been reported to demonstrate limited or no efficacy in brain metastases (2,3).

In addition to chemotherapeutic agents, molecular targeting agents, including erlotinib, have begun to serve an important role in the treatment of lung cancer. Erlotinib is an orally active epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), which is effective against advanced non-small cell lung cancer (NSCLC) with EGFR mutations. Potentially due to their low molecular mass, EGFR-TKIs are able to cross the BBB and have been reported to demonstrate anticancer activity against NSCLC brain metastases by penetrating the BBB (1,2,4). However, the underlying mode of action or molecular mechanisms of metastatic brain lesion resistance to EGFR-TKIs remain unclear. Although immunohistological features of resected lung cancer following EGFR-TKI treatment have been reported in an attempt to elucidate the mechanisms of resistance, no immunohistological study of EGFR status has been performed on brain metastasis while still responding to EGFR-TKI. The present study reports, to the best of our knowledge, the first case of EGFR-mutated NSCLC brain metastasis that was surgically resected while the lesion was responding to erlotinib, and aims to elucidate the mode of response of metastatic brain lesions from EGFR-mutated NSCLC to EGFR-TKIs, and investigate the possible underlying mechanisms involved in drug resistance.

Case report

Case presentation. A 66-year-old Japanese female non-smoker was examined following the complaint of a severe cough. The patient was admitted to Osaka Medical Center for Cancer and Cardiovascular Diseases (Osaka, Japan), a prefectural designated cancer center, in June 2013. Written informed consent was obtained for the analysis described in this report. A chest X-ray revealed a focal mass in the right lung with massive pleural effusion (Fig. 1). Cytodiagnosis of the pleural effusion identified an adenocarcinoma with an L858R EGFR mutation in exon 21. ^{18}F-fluorodeoxyglucose

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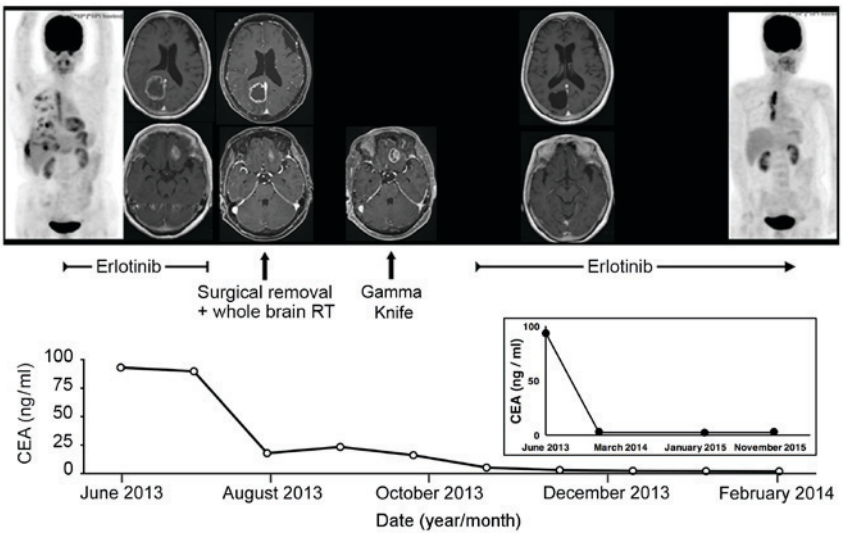

Figure 1. Clinical treatment regime of the case in the present study. The patient was diagnosed with stage IV lung adenocarcinoma (clinical Tumor-Node-Metastasis stage, cT3N1M1b) with an L858R epidermal growth factor receptor mutation in exon 21. Oral administration of erlotinib was initiated during the pre-neurosurgical period in order to restore deteriorated respiratory function. Brain metastasis was surgically resected followed by whole-brain RT and Gamma Knife radiosurgery. Erlotinib maintenance therapy was initiated thereafter. The serum CEA level was maintained within the normal range following initiation of treatment. RT, radiation therapy; CEA, carcinoembryonic antigen.

whole-body positron emission tomography scan revealed brain metastases. Subsequently brain magnetic resonance imaging (MRI) revealed that the lesion had a diameter of >30 mm in the right parietal lobe (Figs. 1 and 2A). The patient was diagnosed with stage IV lung cancer [classified using the clinical Tumor-Node-Metastasis staging system 7th edition (5) as cT3N1M1b].

As severe pleural effusion limited the safety of a neurosurgical procedure aiming to resolve neurological symptoms, including gait disturbance deriving from the metastatic brain lesion, oral administration of erlotinib was initiated during the pre-surgical period in order to restore deteriorated respiratory function. Although erlotinib rapidly regressed pleural effusion and recovered respiratory function, a follow-up MRI 2 weeks following initiation of erlotinib therapy revealed only a slight shrinkage of the brain metastasis (Figs. 1 and 2B). The lesion remained >30 mm and the tumor was surgically resected (Fig. 1C), followed by whole-brain radiation therapy with a total dose of 30 Gy. Gamma Knife radiosurgery was performed on the left frontal lobe lesion followed by erlotinib maintenance therapy. The serum carcinoembryonic antigen level was maintained within the normal range for 2.5 years following initiation of treatment with no evidence of radiological recurrence (Fig. 1).

Pathological findings. Surgical tissue was rapidly fixed in 10% formalin buffer and embedded in paraffin. Extensive necrosis was observed in the tissue with only a slight papillary proliferation at the edge of necrotic tissues (Figs. 2D and 3). Immunohistochemical (IHC) analysis demonstrated that viable cells were positive for thyroid transcription factor 1 and napsin A, confirming that those cells were derived from lung cancer [anti-human thyroid transcription factor 1 (8G7G3/1) and anti-napsin A antibodies; Dako, Agilent Technologies, Inc., Tokyo, Japan; dilution, 1:100; antigen retrieval, microwave heating in 0.1 mol/l sodium citrate solution (pH 6.0)] (Fig. 3) (6). The marker of proliferation Ki-67 labeling index [MIB-1 antibody; dilution 1:20; Immunotech, Marseilles, France; antigen retrieval, microwave heating in 0.1 mol/l sodium citrate solution (pH 6.0)] was low and few viable cells were Ki-67-positive, whereas strong positivity was observed for endothelial cadherin (E-cadherin) [anti-human E cadherin antibody (NHC-38); Dako, Agilent Technologies, Inc.; dilution 1:100; antigen retrieval, microwave heating in 0.1 mol/l sodium citrate solution (pH 6.0)] (Fig. 3).

Subsequently, the EGFR mutation status was evaluated using IHC analysis. Tissues were blocked in goat serum for 1 h at 37˚C and incubated with primary antibodies (Cell Signaling Technology Japan, K.K., Tokyo, Japan; dilution, 1:100) at 4˚C overnight. Antibodies specific for L858R-mutant EGFR (43B2), exon 19-deleted EGFR (E746-A750del; D6B6) and pan-EGFR (D38B1) were used. All specimens were recorded using a Virtual Slide image capturing system (Olympus Corporation, Tokyo, Japan). In contrast with expectations, no EGFR-positive staining was observed in the E-cadherin-positive viable cells (Fig. 3).

Genetic analysis. In addition to IHC examination, genetic examination of the mutation status of the EGFR gene was performed. Fluorescence resonance energy transfer-based preferential homoduplex formation assay (F-PHFA) technology was used as previously described (7). This technique relies on the theory that labeled double-stranded DNA (dsDNA) and a non-labeled amplicon conduct competitive hybridization under a slow thermal gradient. A strand-exchange reaction takes place only when the labeled dsDNA and non-labeled amplicon are 100% identical in length and sequence, which causes the labeled dsDNA to be diluted by the excess amount of amplicon and emits a fluorescent signal led by the separation of the strand with the fluorescent molecule and the strand with the quenching molecule (7).

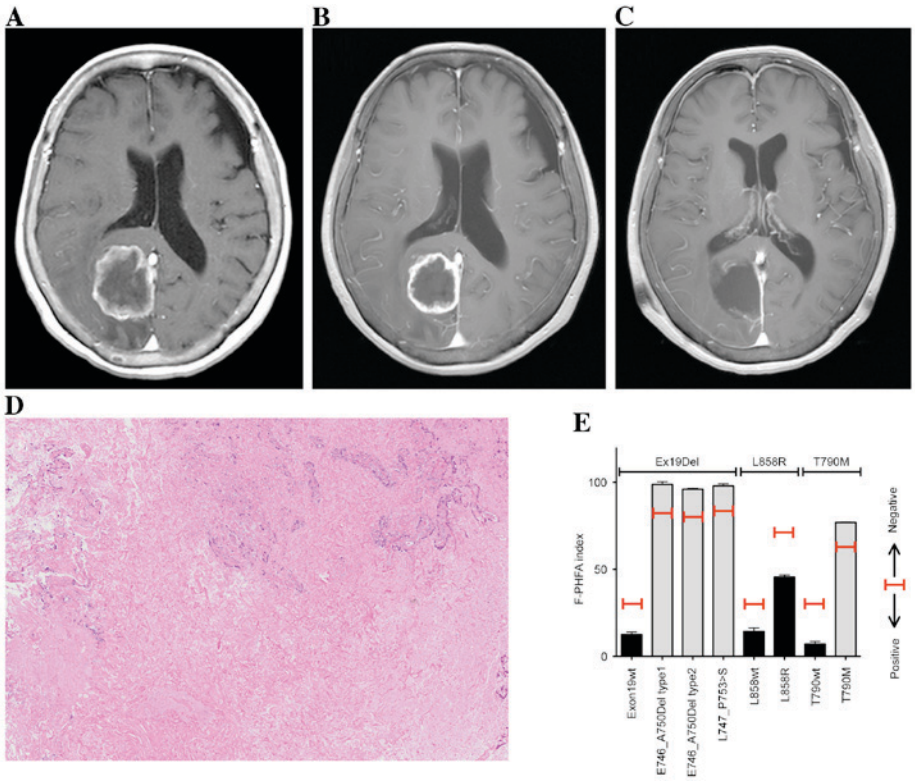

Figure 2. Brain magnetic resonance imaging during the course of treatment and macroscopic view with F-PHFA gene analysis of the removed tissue. Brain magnetic resonance imaging of (A) pretreatment, (B) 2 weeks following erlotinib treatment with marked shrinkage of the tumor and (C) post-surgery achieving gross total resection of the tumor. (D) Necrosis was observed with slight papillary proliferation at the edge of necrotic tissues (magnification, x40). (E) For F-PHFA gene analysis, positive signals are defined by F-PHFA indexes lower than the threshold, indicated by red lines. The EGFR mutation status was Ex19Del-negative, L858R mutation-positive and T790M mutation-negative. F-PHFA, fluorescence resonance energy transfer-based preferential homoduplex formation assay; Ex19Del, exon 19 deletion; wt, wild-type.

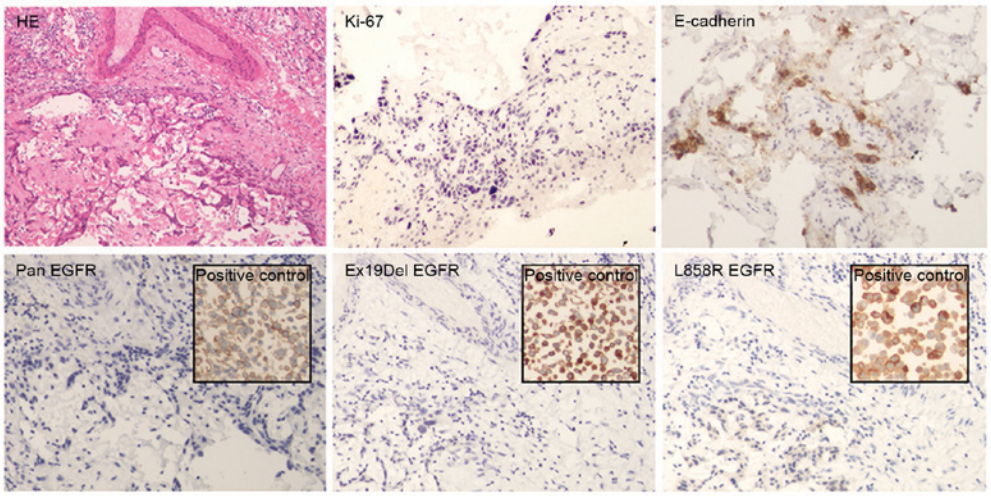

Figure 3. Immunohistochemical staining of the resected tissue. Tissue was stained using HE, Ki-67, E-cadherin, pan-EGFR, exon 19-deleted EGFR and L858R mutant EGFR. Representative images of low Ki-67 labeling index, positive E-cadherin expression and negative EGFR expression. All images are presented at a magnification of x200. HE, hematoxylin and eosin; Ki-67, marker of proliferation Ki-67; E-cadherin, endothelial cadherin; EGFR, epidermal growth factor receptor; Ex19Del, exon 19 deletion.

This assay demonstrated that the tissue possessed the L858R EGFR mutation, whereas exon 19 deletion was not detected. The presence of the T790M EGFR mutation, a secondary EGFR mutation that is considered significant for EGFR-TKI tumor resistance (8,9), was also assessed, revealing that the tissue did not have the T790M mutation (Fig. 2E).

Discussion

Previous case reports have advocated the efficacy of EGFR-TKI for patients with brain metastasis arising from EGFR-mutated lung cancer (10,11). Berger *et al* (11) reported that the response rates using EGFR-TKI in brain metastasis

with EGFR mutation were <80%, which is markedly high when the expected response rate for conventional treatments such as whole-brain radiation therapy is taken into account. Although the concentration of erlotinib in the cerebrospinal fluid is reported to be decreased compared with that in the plasma (3), a disrupted BBB around the metastatic lesion is considered to lead to an increase in the local drug concentration (2). Consistent with previous studies (1,12,13), pre-operative MRI in the present study demonstrated a slight but notable regression of brain metastasis following initiation of EGFR-TKI therapy (Figs. 1 and 2).

Despite a marked amount of data associated with the clinical response of EGFR-mutated lung cancers to EGFR-TKIs (11,12), there is a lack of evidence of the histopathological changes in cancer tissues following EGFR-TKI treatment. A previous study by Lara-Guerra *et al* (14) demonstrated the histopathological features of lung cancer tissues treated with gefitinib. In the previous study, EGFR-mutated tumors following EGFR-TKI therapy demonstrated decreased tumor cellularity and a decreased Ki-67 labeling index within the fibrous stroma compared with wild-type EGFR adenocarcinoma tumors. Furthermore, the study described that histological features were associated with the clinical response of the tumor to EGFR-TKI (14). In the present study, viable metastatic cells existed as foci within a large necrotic area. It was also confirmed that those viable cells presented decreased proliferative activity evaluated using Ki-67 labeling similar to the study by Lara-Guerra *et al* (14). These results confirm that EGFR-TKI demonstrates cytotoxic action against brain metastatic lesions, potentially beyond the BBB.

Furthermore, EGFR mutation status during treatment with EGFR-TKI was evaluated using IHC and F-PHFA gene analysis. IHC demonstrated that viable cells did not express L858R mutant EGFR, whereas the use of F-PHFA demonstrated that these cells did have the L858R EGFR mutation. Although viable cells that are resistant to EGFR-TKI treatment possessed the L858R EGFR mutation, EGFR expression was potentially markedly downregulated in those cells. This model is also supported by the fact that these cells demonstrated negative staining when anti-pan-EGFR antibodies were used for immunostaining. The secondary EGFR mutation T790M, which is observed in ~50% of EGFR-TKI-resistant patients (8), is one of the primary mechanisms that is considered important in the development of EGFR-TKI resistance. The T790M EGFR mutation was also evaluated using F-PHFA, demonstrating that viable cells were negative for the T790M EGFR mutation. It is hypothesized that the T790M mutation occurs during EGFR-TKI therapy and may exist prior to treatment (11,15). EGFR-TKI-resistant brain metastatic lesions have been reported to have the T790M EGFR mutation (16), similar to primary cancer (8). The present study is markedly distinct from previously reported cases in that the tumor was resected and evaluated prior to the lesion demonstrating clinical resistance to EGFR-TKI. The results of the present study demonstrate that viable cells, although possessing reduced proliferative capability, are present while the entire lesion responds to EGFR-TKI. Furthermore, to the best of our knowledge, the present study is the first to provide evidence that EGFR-mutated NSCLC cells are able to evade the cytotoxic effect of EGFR-TKI by downregulating EGFR expression, without exhibiting the T790M EGFR mutation.

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