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Case report

Successful sequential treatment of refractory tumors caused by small cell carcinoma transformation and EGFR-T790M mutation diagnosed by repeated genetic testing in a patient with lung adenocarcinoma harboring epidermal growth factor receptor mutations: A case report

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ABSTRACT

NSCLC patients with EGFR mutations respond to EGFR-TKIs; however, the management of refractory tumors to EGFR-TKIs remains unclear. We demonstrated that repeated genetic testing might be useful for detecting resistance mechanisms as well as for decision-making in EGFR mutated NSCLC patients, following the emergence of resistance to the initial EGFR-TKIs.

A 69-year-old man was diagnosed with lung adenocarcinoma with an EGFR exon 19 deletion. After tumor regrowth treated with erlotinib and chemotherapy, he was diagnosed with an SCLC transformation and administered chemotherapy to treat the SCLC. After the resistance of chemotherapy, the EGFR-T790M mutation by liquid biopsy was detected and treated him with osimertinib, which resulted in a clinical response.

1. Introduction

Treatment with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) show beneficial outcomes in non-small cell lung cancer (NSCLC) harboring EGFR mutations [1]; however, nearly all tumors eventually acquire resistance to this class of compounds. Of them, the gatekeeper EGFR-T790M mutation is the most frequent, occurring in half of tumors with acquired resistance to first and second generation of EGFR-TKIs. Recently, third generation of EGFR-TKI osimertinib was developed to overcome acquired resistance by EGFR-T790M mutations. However, it is one of the critical issues to detect less EGFR-T790M mutations by genetic testing after resistance to initial EGFR-TKI [2]. Transformation to small cell lung cancer (SCLC) is one of the acquired resistance mechanisms to EGFR-TKIs, accounting for 4–10% of instances [3,4].

Although previous reports have suggested systemic chemotherapy targeting SCLC to be effective in cases of SCLC transformed from adenocarcinoma harboring EGFR mutations [5,6], the therapeutic strategy for cases refractory to chemotherapy for SCLC transformed remains unknown.

Here we report a case of adenocarcinoma harboring EGFR exon 19 deletion mutations that transformed to SCLC following EGFR-TKI treatment. After resistance to chemotherapy targeting the SCLC components had developed, we detected an EGFR-T790M mutation by repeated genetic testing and treated the patient with osimertinib. Our case report revealed the importance of repeated genetic testing for the detection of the EGFR-T790M mutation as well as for decision-making regarding therapeutic strategies in NSCLC patients with EGFR mutations.

2. Case report

A 69-year-old man whose chest X-ray findings indicated an upper left lung nodule was admitted to our hospital in June 2014. Further radiological examinations revealed a 26-mm nodule in the upper lobe of the left lung, a metastatic brain tumor in the right parietal lobe, and multiple bone metastases. We diagnosed the patient with lung adenocarcinoma with an EGFR exon 19 deletion following bronchoscopy of the upper lobe of the left lung. His clinical stage was T1bN2M1b stage IV. He underwent gamma knife for the non-symptomatic brain metastasis in July 2014. After that, he was administered an alternating therapy comprising erlotinib and systemic chemotherapy, combined

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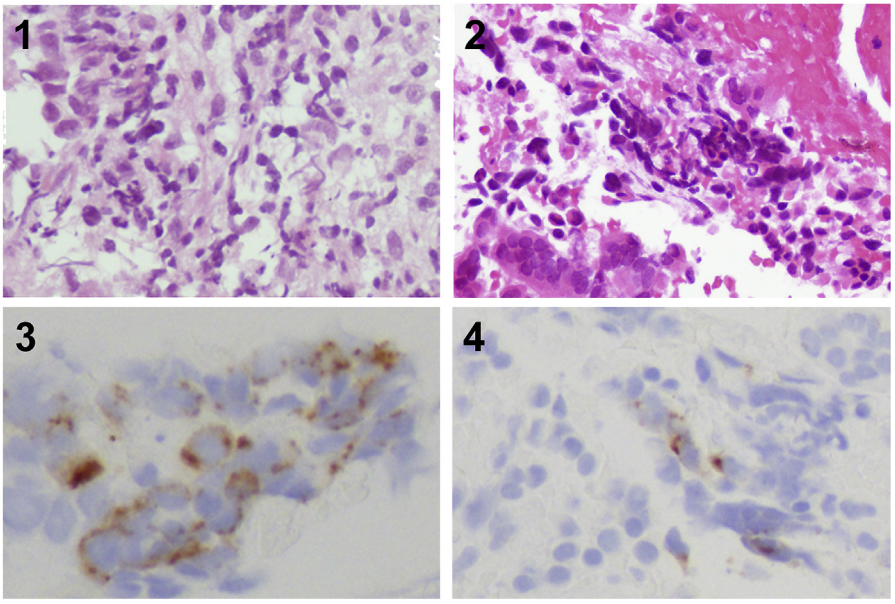

Fig. 1. Pathological findings **(A)** The first biopsy sample revealed adenocarcinoma in the left lower lobe of the primary lesion (hematoxylin and eosin staining ×400). **(B)** The second biopsy sample revealed small cell lung cancer in the left lower lobe of the primary lesion (hematoxylin and eosin staining ×400). **(C, D)** Immuno histopathological analysis showed positive staining for chromogranin **(C)** and synaptophysin **(D)** in the second biopsy sample (×400).

therapy with pemetrexed plus bevacizumab for four cycles, followed by maintenance treatment with pemetrexed plus bevacizumab; this treatment showed a clinical response for 25 months. However, positron emission tomography-computed tomography (PET-CT) imaging in September 2016 showed that the primary tumor has increased in size and that a right adrenal metastasis newly emerged. We subsequently performed re-biopsy of the primary tumor in October 2016 and found both an adenocarcinoma harboring an EGFR exon 19 deletion without EGFR-T790M mutation and an SCLC component that showed positive staining for chromogranin and synaptophysin in the biopsy specimens (Fig. 1). The patient was administered four cycles of combined chemotherapy with cisplatin and irinotecan targeting the sub-population of SCLC from October 2016. Although the tumors showed a partial response with this therapy for five months, they worsened again and elevated the blood levels of carcinoembryonic antigen (CEA) but not neuron-specific enolase (NSE) or pro-gastrin-releasing peptide (ProGRP), in February 2017. A liquid biopsy revealed only the EGFR exon 19 deletion without the EGFR-T790M mutation. From April 2017, the patient was administered the second-generation EGFR-TKI afatinib (40 mg/day) targeting the residual component of the adenocarcinoma. After a month of medication, CT imaging revealed that the primary tumor and the mediastinal lymph node metastasis had reduced in size and were maintained for three months. After this time, a second liquid biopsy was performed when tumors relapsed and the EGFR exon 19 deletion and EGFR-T790M mutation were detected. The patient received the third-generation EGFR-TKI osimertinib (80 mg/day) from July 2017 and maintained a clinical response for 10 months (Fig. 2).

3. Discussion

One of the critical issues for treatment with EGFR-TKIs is that almost all tumors confer various resistance systems; thus, it is necessary to develop a novel therapeutic strategy for tumors following the emergence of resistance to the initial EGFR-TKIs. There are two hypotheses for the pathogenesis of SCLC transformation after exposure to EGFR-TKIs in NSCLC patients with EGFR mutation [8]. One hypothesis is that small populations with SCLC are present in the pre-treated tumor. As the adenocarcinoma component is successfully treated with EGFR-TKIs, the SCLC component finally becomes dominant and is detected by re-biopsy. The other hypothesis is the histological transformation of EGFR-mutant adenocarcinoma to *de novo* SCLC during EGFR-TKI treatment. Matthew et al. reported that the inactivation of both RB1 and TP53 caused adenocarcinomas harboring EGFR mutations to transform to SCLC, consistent with clinical specimens [9]. The clinical course of the present case supports the latter hypothesis based on the original tumor specimens which included only adenocarcinoma components; thus, the components of SCLC might emerge and become the majority of tumors. Interestingly, Watanabe et al. reported that chromogranin and synaptophysin were positively stained in the portion of adenocarcinoma even in before EGFR-TKI treatment [7].

There is no evidence for the treatment of refractory tumors after chemotherapy for SCLC transformed from NSCLC harboring EGFR mutations. Our case showed that blood CEA levels were elevated during chemotherapy treatment for the transformed SCLC. We observed increased adenocarcinoma components without EGFR- T790M mutations and chose to treat with afatinib. After the tumors became refractory to afatinib, we detected the EGFR-T790M mutation by liquid biopsy and started osimertinib treatment as the third choice of EGFR-TKI. Although several cases have reported refractory tumors, including the coexistence of EGFR-T790M mutation and SCLC transformation after acquiring resistance to EGFR-TKI [10–12], this is the first report of metachronous resistances to targeted agents diagnosed by repeated genetic testing and successful sequential therapies for the management of tumor growth. Moreover, to our knowledge, this is also the first report of the detection of EGFR-T790M mutation by liquid biopsy after refractory tumors with SCLC transformation.

Therefore, our case report indicated the importance of repeated genetic testing for the detection of EGFR-T790M mutation even after tumors have undergone SCLC transformation.

There are two limitations in this case report. The first limitation is the possibility that the biopsy specimens might have been heterogeneous. Although the SCLC component was not detected in the initial biopsy samples, both SCLC and adenocarcinoma were detected in re-biopsy. In addition, it might be possible for the T790M mutation to be present in different lesions or primary lesions other than the biopsied site [13–15]. However, we believe that the mutation might have increased in prevalence or emerged following afatinib treatment because the T790M mutation was detected only in the second liquid biopsy after four months of afatinib treatment. The other limitation is the sensitivity of liquid biopsy. In our case, we used the Roche cobas® EGFR mutation test v2, which is approved by Japanese insurance [16]. Although the probability of matched detection of EGFR mutations between tissue biopsy and liquid biopsy is 91%, Weber reported 11 of 28 samples (~40%) with EGFR mutation detected by tissue biopsy but not by

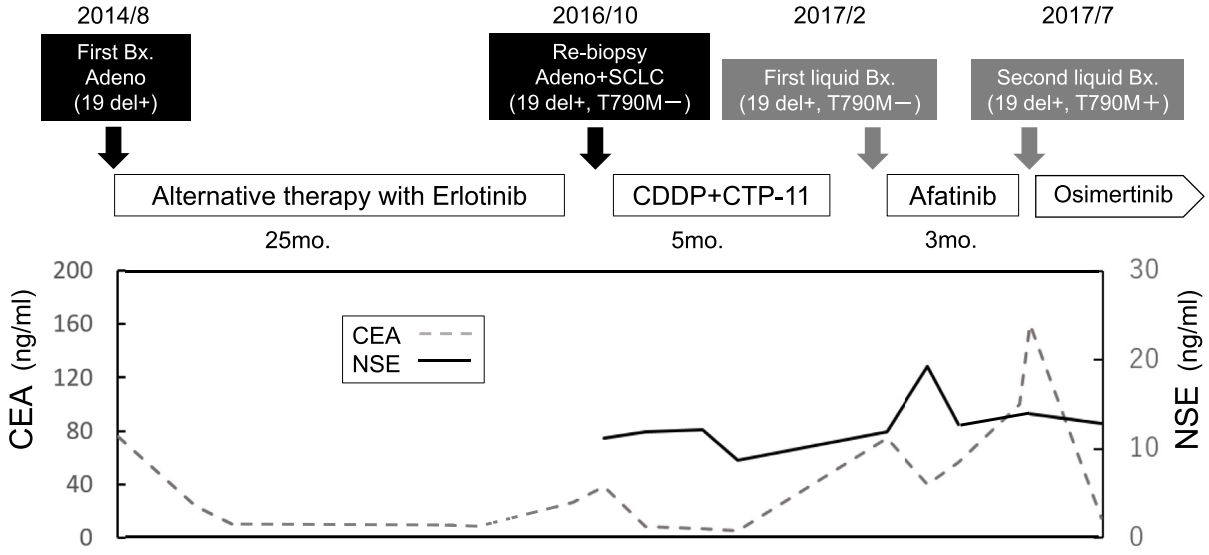

Fig. 2. Clinical course of the sequential treatment and tumor markers for EGFR-mutated NSCLC. An alternating therapy is composed of erlotinib and systemic chemotherapy, combined therapy with pemetrexed plus bevacizumab. Bx., biopsy; 19 del, exon 19 deletion in EGFR; T790M, EGFR-T790M mutation.

liquid biopsy [17]. Therefore, it is possible that the EGFR-T790M mutation existed as a minor clone from the first liquid biopsy that detected only the EGFR exon 19 deletion. Further experiments are needed to resolve the discrepancy between tissue samples and alternative specimens.

4. Conclusion

In this case report, repeated biopsies and genetic testing contributed to appropriate sequential therapy after the acquired resistance to the initial EGFR-TKI, even if tumors transformed to SCLC. It might be a useful tool for the detection of resistance mechanisms to therapeutic agents as well as for decision-making regarding subsequent therapeutic strategy in NSCLC patients with EGFR mutations. These findings encourage us to perform repeated biopsies regardless of tissue and liquid methods following acquired resistance to several EGFR-TKIs.

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Conflicts of interest

The authors declare no conflicts of interest associated with this manuscript.

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