Case Report
Primary gefitinib resistance in NSCLC with EGFR combined RB1 mutation: a case report

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Received November 18, 2018; Accepted May 7, 2019; Epub July 15, 2019; Published July 30, 2019

Abstract: Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) act as a standard and effective first-line treatment of non-small cell lung cancer (NSCLC) patients with classical EGFR mutations. However, primary or acquired resistance to TKIs remains a major clinical problem. Here, a case of a 61-year-old NSCLC patient with a combined EGFR and retinoblastoma 1 (RB1) gene mutation is reported. The patient exhibited sensitivity to chemotherapy but primary resistance to EGFR-TKIs. This case with a rare EGFR mutation deepened our understanding of EGFR-TKI resistance and provided insight into precise clinical diagnosis that would benefit NSCLC patients from targeted therapies.

Keywords: Epidermal growth factor receptor (EGFR), mutation, gefitinib, non-small cell lung cancer, RB1

Introduction
Lung cancer is the most common malignancy and leading cause of cancer death worldwide [1]. NSCLC is the largest histological subtype of lung cancer, which accounts for approximately 85-90% of all lung cancers [2]. With development of molecular biology, targeted therapy and driver mutations have achieved remarkable success. With the function of blocking the activation of epidermal growth factor receptor (EGFR) downstream signaling, EGFR tyrosine kinase inhibitors (TKIs) are effective treatments for NSCLCs with EGFR mutations. EGFR-TKI therapy has been recommended as the first-line treatment for EGFR-mutated advanced lung adenocarcinoma. Although most patients initially benefit from TKI therapies, they inevitably develop resistance. This case involves a NSCLC patient with an EGFR sensitive mutation who exhibited sensitivity to chemotherapy and primary resistance to EGFR-TKI. Next-generation sequencing (NGS) analysis and immunohistochemistry (IHC) staining revealed the co-occurring RB1 deletion and EGFR mutation.

Case presentation
A 61-year-old female, without a smoking history, presented with a fever and aggravated cough with sputum. Chest computed tomography (CT) scan indicated a pulmonary malignant tumor in the superior lobe of the right lung (Figure 1A). The patient underwent surgery in June 2016. Hematoxylin and eosin (H&E) staining showed a typical morphology of invasive lung adenocarcinoma (T3N1M0, stage IIIA). Two months after the operation, the Magnetic Resonance Imaging (MRI) enrichment indicated asymptomatic brain metastasis in the sub-cortex of the right frontal lobe (Figure 1B). Further PET-CT showed lymphatic metastasis in the right-sided sub-pleural lymph nodes (SUV=3.7) and supraclavicular lymph nodes (SUV=5.4) (Figure 1C). Subsequently, the patient underwent two cycles of radiotherapy treatment with a combination of pemetrexed (800 mg), cisplatin (110 mg) and bevacizumab (400 mg). NGS (OrigMed, Shanghai, China) analysis on the tumor tissue identified a L858R mutation in exon 21 of EGFR.
Figure 1. CT scan (A) CT scan showed a space occupying focus (arrow) in the superior lobe of the right lung. (B) Cranial MRI enrichment showed abnormal strengthening signals (arrow) in the sub-cortex of the right frontal lobe. (C) PET-CT showed high metabolic changes in the right-sided sub-pleural lymph nodes.

Figure 2. CT scan after radiotherapy combined with chemotherapy. A. Cranial MRI did not show intracranial metastasis. B. Chest CT indicated the right-sided sub-pleural nodules (arrow) had shrunk dramatically. C. Chest CT showed the enlarged right-sided sub-pleural nodules (arrow) after receiving gefitinib treatment for 20 days. D. Chest CT showed the enlarged right-sided subpleural nodules (arrow) after receiving gefitinib treatment for two months.

Cranial MRI showed no intracranial metastasis (Figure 2A) and B-ultrasound indicated no enlargement of the right supraclavicular lymph nodes upon reexamination on September 28, 2016. Meanwhile, dramatic shrinkage of the right-sided sub-pleural nodules were detected by chest CT (Figure 2B). Two more cycles of treatment were adopted following the original chemotherapy plan in October 2016. Since the patient could not tolerate the planned radiotherapy after four cycles of intensive chemotherapy involving the combination of three drugs, a single 250 mg oral dose of gefitinib treatment was adopted on November 1, 2016.

A second chest CT in November 2016 showed enlarged right-sided sub-pleural nodules (Figure 2C). Since the Response Evaluation Criteria in Solid Tumors (RECIST) was still within the range of stable disease (SD), gefitinib treatment was continued. A third chest CT in December 2016 showed soft tissue shadows in
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Figure 3. Immunohistochemistry (IHC) staining of tumor samples. IHC showed positive signals for EGFR L858R (A) and RB1 protein (B) (200 x magnification).

the pleura and chest wall, as well as multiple dramatically enlarged lymph nodes in the mediastinum (Figure 2D), right subclavian and right sub-axillary. RECIST disease progression (PD) for the patient indicated the failure of gefitinib in maintenance therapy. The single agent pemetrexed (800 mg/m²) chemotherapy was adopted on January 17, 2017. Unfortunately, the patient developed jaundice and abnormal liver function and died on February 1, 2017.

To explore the cause of acquired gefitinib resistance, the former tumor tissues were subjected to a comprehensive NGS panel (Origimed, Shanghai, China) analysis. Besides the L858R EGFR mutation, the RB1c.381-23_381del mutation was also detected. Immunohistochemistry (IHC) also supported the positive signals for EGFR L858R (Figure 3A) and the inactivation of RB1 protein (Figure 3B).

Discussion

Overall survival (OS) of most patients with NSCLC is less than one year even if platinum-based combination chemotherapy is used [3]. Compared with traditional platinum-based combination chemotherapy, EGFR-TKI harbors a better clinical benefit in the treatment of NSCLC patients with EGFR mutations. Lee et al. demonstrated that the first-generation EGFR-TKIs (gefitinib and erlotinib) improved progression-free survival (PFS) compared to platinum-based combination chemotherapy [4]. As a second-generation EGFR-TKI, afatinib is associated with more improved PFS compared to chemotherapy used in first-line therapy of EGFR-mutant NSCLC [5, 6] and also demonstrated improved PFS compared to gefitinib in treatment-naive patients [7]. Compared to cisplatin-based chemotherapy, afatinib also led to an OS benefit in a subset of lung adenocarcinoma patients with exon 19 deletions [8]. Dacomitinib, a second-generation EGFR-TKI, was superior to gefitinib with an improved PFS in first-line treatment of EGFR mutant NSCLC [9]. Osimertinib, a third-generation EGFR-TKI, is an oral agent that was approved by the Food and Drug Administration (FDA) in November 2015 for the treatment of NSCLC patients with metastatic EGFR T790M mutations [10]. A phase III AURA3 (NCT02151981) trial, in which 419 patients with EGFR T790M-positive lung cancer were randomly assigned to osimertinib or platinum therapy plus pemetrexed, demonstrated that osimertinib was significantly superior to chemotherapy in objective response rate (ORR) and PFS [11]. Also, in the treatment of 144 patients with stable and asymptomatic brain metastases in the phase III AURA3 trial, osimertinib treatment had a longer PFS than treatment with chemotherapy [11].

Even though treatment with EGFR-TKIs confers clinical benefit compared to platinum-based combination chemotherapy, patients treated with EGFR-TKIs, including osimertinib, will unavoidably develop acquired resistance after 9-14 months of PFS [12, 13]. Various mechanisms of resistance to TKIs have been identified, including the secondary EGFR mutation, the activation of EGFR parallel signaling pathways and combined mutations with other genes [14, 15]. T790M mutation in the 20th exon of EGFR, which accounts for 40% of all EGFR mutations, is the most common mechanism of resistance to first generation EGFR-TKIs.

Histological transformation might also contribute to the EGFR-TKI resistance. Sequist et al.
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[16] found that this small cell histological transformation developed resistance to EGFR-TKIs in EGFR mutated NSCLC patients. Importantly, the small cell lung cancer (SCLC) transformed tumor retained the original activating EGFR mutation, suggesting their NSCLC origin.

Additionally, SCLC transformed from EGFR-mutant NSCLC is thought to share the same origin as primary SCLC in pathogenesis, which may help identify the resistance mechanism of TKIs by transformation. Pao et al. [17] and Brose et al. [18] found that the mutations in EGFR and KRAS are common in pulmonary adenocarcinoma, while the RB1 deletion is observed in almost all SCLC cases [19]. Repeat biopsy studies of resistant EGFR mutant patients consistently revealed that cases involving small cell histological transformation had a RB1 deletion rate of 100%, but was rarely found in the cases that remained NSCLC [20]. Loss of RB1 in EGFR-TKI resistant patients is vital to small cell histological transformation, and EGFR-TKIs might promote this pathological transition by inducing RB1 deletion [21].

Here, the case harboring the L858R mutation of EGFR showed primary resistance to gefitinib, but was not sensitive to EGFR-TKIs as expected. NGS analysis indicated that both the RB1 deletion and EGFR mutation occurred in the same tumor sample before gefitinib treatment. From these findings, it can be speculated that the co-mutation of EGFR and RB1 might directly contribute to EGFR-TKI resistance. Thus, detection of a single driver mutation in lung cancer patients might not be enough for clinical diagnosis and treatment. Comprehensive genetic tests are needed to aid in the selection of patients who may benefit from targeted therapies.

The detection of driver mutations in NSCLC and the corresponding treatment of TKIs has become the routine diagnosis and treatment procedure. Therefore, providing personalized therapy to patients is an urgent issue that needs to be addressed in the clinic. This report provided a rare case of primary resistance to EGFR-TKIs in a NSCLC patient with EGFR and RB1 mutations, and will potentially contribute to the development of personalized treatment of NSCLC.

Acknowledgements

The current study was supported by grant from Three-year Action Plan of Development of Traditional Chinese Medicine in Shanghai (grant no. ZY3-LCPT-2-1001).

Disclosure of conflict of interest

Qiang Cui is the employee of OrigiMed, and the other authors have no conflict of interest to disclose.

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