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CASE REPORT

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Primary resistance to crizotinib treatment in a non-small cell lung cancer patient with an EML4-ALK rearrangement: a case report

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ABSTRACT Crizotinib, a small molecular tyrosine kinase inhibitor, manifests dramatic responses in patients with non-small cell lung cancer with echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase (EML4-ALK) rearrangements. ALK gene point mutation is the primary mechanism of acquired crizotinib resistance; however, the intrinsic mechanism is not fully understood. Here, we report a patient with a low mutant allele fraction (MAF) of EML4-ALK rearrangement, who experienced primary resistance to crizotinib treatment. The patient was a 66-year-old Chinese man, who had a history of metastatic lung cancer and was treated with first- and third-generation epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs). After 14 months of osimertinib treatment, his disease progressed, and next-generation sequencing was performed from a liquid biopsy of the patient's blood. An EML4-ALK rearrangement was found and crizotinib was administered. The patient's lung lesions continued to progress after one month of crizotinib treatment, and pemetrexed-bevacizumab was initiated. After two cycles of chemotherapy, the metastatic cancers shrunk, and the patient maintained stable disease at his last follow-up. EML4-ALK rearrangements can happen in patients with EGFR-positive NSCLC, after acquired resistance to EGFR TKI treatment. The EGFR T790M and C797G mutations occur in cis is a critical mechanism of resistance to osimertinib therapy. The MAF of EML4-ALK rearrangements in cancer cells might be a predictive factor for crizotinib treatment.

KEYWORDS Non-small cell lung cancer; EML4-ALK; target therapy; crizotinib

Introduction

The epidermal growth factor receptor (EGFR) is a transmembrane protein, and upon mutation, is a key driver gene in non-small cell lung cancer (NSCLC). In Southeast Asia and China, patients with lung cancer frequently have high rates of EGFR mutations, especially non-smoking women[1-3]. EGFR tyrosine kinase inhibitors (EGFR TKIs), which reversibly interact with the ATP binding pocket of the kinase and inhibit cancer proliferation and metastasis, have shown promising results in patients with EGFR mutation-positive NSCLC. Patients who are treated with gefitinib or erlotinib usually acquire resistance, with median progression-free survival (PFS) durations of 10 to 14 months[4,5]. About 50%–60% of acquired resistances are induced by the EGFR T790M mutation, which is a second EGFR point mutation that involves a threonine to methionine amino acid substation at position 790 (T790M), is often detected in tumor cells after disease progression. This mutation enhances EGFR affinity for ATP, reduces the ability of ATP-competitive and reversible EGFR TKIs to bind to the EGFR tyrosine kinase domain, and results in cancer cell resistance to gefitinib and erlotinib[6]. The EGFR T790M mutation is the most common cause of acquired drug resistance for first-generation EGFR TKIs. Monotherapy with osimertinib has been associated with a response rate of 61% and a PFS of 9.6 months, with limited skin and gastrointestinal adverse effects, among patients with EGFR T790M-positive cancers[7]. The mechanisms of resistance to osimertinib or other third-generation EGFR TKIs are very complicated, and the reported mutation sites and/or rates have substantially varied among related studies. Phenotypic transformation, new EGFR point mutation, targets loss, or pathways activation

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(c-Met amplifications, EML4-ALK rearrangement, etc.) have been the most frequent causes of acquired resistance[8,9].

Patients with EML4-ALK rearrangements often manifest dramatic responses to crizotinib and acquire resistance, with progression free survivals of about 11 months[10-13]. ALK gene point mutations are the primary mechanism of crizotinib acquired resistance; however, the intrinsic mechanism is poorly understood[14]. Here, we report on a patient with NSCLC with an EML4-ALK rearrangement who experienced primary resistance to crizotinib treatment.

Case report

The patient was a 66-year-old Chinese man, who was admitted to our hospital for coughing and blood spiting that lasted for a month. A chest computer assisted tomography (CAT) scan showed a 2.5 × 2.5 cm round node on his left upper lung (**Figure 1A**). A lobotomy of the left lung and mediastinal lymph node resections were performed on November 29, 2012, and a stage IIIA (T1N2M0) lung adenocarcinoma that metastasized to the left hilar and mediastinal lymph nodes was histologically confirmed. Four cycles of adjuvant chemotherapy with cisplatin and pemetrexed were administered, four weeks after surgery. Eighteen months later, the patient complained of right upper abdominal pain, and a positron emission tomography/computed tomography (PET/CT) scan showed a 3 × 3 cm mass on the liver and 1.5 × 1.5 cm node on the right adrenal gland (**Figure 1B**). A core needle biopsy of the liver lesion was performed, and a diagnosis of metastatic lung adenocarcinoma was made by a pathologist. The EGFR gene panel was examined, in the biopsied tissue, by Amplification Refractory Mutation System polymerase chain reaction (ARMS PCR), and an EGFR 19 del was detected. Gefitinib was administered, and the patient achieved a partial response. After 18 months of gefitinib treatment, the patient complained of right leg pain, and his abdominal CAT scan showed multi-peritoneal lymph nodes metastases. A bone scan showed right femur metastasis (**Figure 1C**), and femoral head replacement to prevent femoral fracture was performed in December 2015. Bone metastasis was confirmed by the pathologist, and the EGFR T790M mutation was identified in the bone tissue. Osimertinib was then administrated, and the patient achieved a partial response. Fourteen months after remission, his lung disease progressed (**Figure 1D**). Next-generation sequencing (NGS) was performed on the patient's blood, and of the 1021 genes that were tested, four genetic point mutations and an EML4-ALK rearrangement were found. The mutant allele fractions (MAFs) for EGFR 19 del, T790M, C797G, and PIK3CA were 2.8%, 4.6%, 2.6%, and 0.9%, respectively, and the EML4-ALK rearrangement rate was 0.9%. Because the EGFR T790M and C797G mutations occurred in cis, which would generate resistance to all EGFR TKIs, crizotinib (Xalkori, Pfizer, USA) was administered. After one month of crizotinib treatment, the patient's condition further deteriorated, and his chest CT scan showed disease progression (**Figure 1E**). Chemotherapy with pemetrexed and bevacizumab was initiated, and the patient maintained stable disease at his last follow-up on December 20, 2017 (**Figure 1F**).

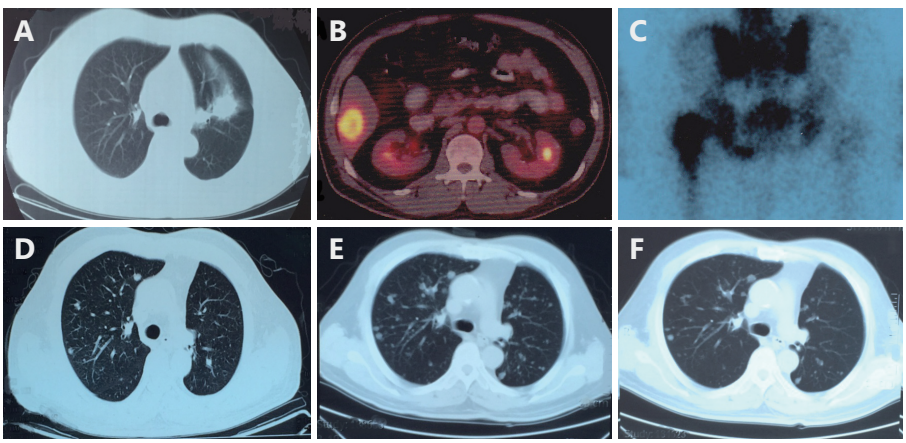

Figure 1 (A) A CAT scan showed a 2.5 × 2.5 cm, round node on the left upper lung and an irregular mediastinal lymph node. (B) A PET-CT scan showed liver metastasis. (C) A bone scan showed femoral metastasis, after 18 months of gefitinib treatment. (D) New lesions occurred after 14 months of osimertinib treatment. (E) Disease progression after crizotinib therapy for one month. (F) Stable disease, after three cycles of pemetrexed plus bevacizumab therapy.

Discussion

Adenocarcinoma and large cell carcinoma account for 50%–60% of lung cancers. About 50% of Eastern Asian and 15% of Caucasian patients with non-squamous NSCLC harbor EGFR mutations[1,15]. Furthermore, 4%–7% of patients with NSCLC have EML4-ALK rearrangements, and ROS1 rearrangements and BRAF V600 mutations occur at lower frequencies[16,17].

Previous studies have established EGFR TKIs as the mainstay treatment for patients with NSCLC harboring EGFR activating mutations[18]. However, there is an eventual loss of TKI efficacy due to development of acquired resistance. The EGFR T790M mutation is the leading cause for resistance to first generation EGFR TKIs, but for third generation EGFR TKIs, the resistance mechanism is much more complicated. Hence, the discovery of new drugs that can overcome resistance to third generation EGFR TKIs is of critical importance for prolonging the survival of patients with NSCLC[19,20].

The EML4-ALK translocation involves a fusion of the N - terminus of EML-4 with the kinase domain of ALK, to produce the EML4-ALK fusion gene. A series of research has shown that this translocation involves several variations, all of which lead to catalytically active kinase fusion protein variants, and experiments in animal models have proven that the kinase activity is required for carcinogenicity[21,22]. ALK rearrangement-positive advanced NSCLC has had high response rates to crizotinib treatment; however, the exact mutation allele fraction (MAF) threshold that is needed to achieve a treatment response has not been reported in English[23]. In this case, the patient's lung lesions progressed quickly after the initiation of crizotinib treatment. This might be because the patient harbored an EGFR 19 del, T790M mutation, and C797G mutation, as primary tumor driver genes, or because the EML4-ALK rearrangement MAF was too low.

For patients harboring EGFR T790M and C797G mutations, we must distinguish whether the two mutations occurred *in cis* or *in trans*. In this patient, three EGFR point mutations occurred *in cis*, which indicated that the patient would be resistant to all EGFR TKIs[24,25]. Previous data has shown that checkpoints inhibitors had limited effects on EGFR-mutated lung cancers, because the tumor cells either lacked PD-L1 or expressed PD-L1 at low levels[26-28]. Chemotherapy was the optimal choice, and low toxicity drugs with pemetrexed were used, since the patient had a long treatment history, and his performance status (PS), according to the Eastern Cooperative Oncology Group (ECOG) scoring system, was 2.

The formation of new blood vessels, known as angiogenesis, is a fundamental event in the process of tumor growth and metastatic dissemination. The vascular endothelial growth factor (VEGF) and its receptors play an essential role in tumor proliferation[29-31]. Bevacizumab, a recombinant humanized monoclonal antibody, combined with VEGFA, attenuates VEGFA-dependent tumor blood vessels formation, normalizes tumor blood vessels, prompts tumor cell apoptosis, and shrinks tumors. This patient presented multi-organ metastasis, in which the VEGF signaling pathway might play an important role.

Conclusions

The EGFR T790M and C797G mutations occurred in cis were the critical mechanisms of resistance to osimertinib. The EML4-ALK rearrangement was found in this patient with EGFR-positive NSCLC after acquired resistance to EGFR TKI treatment. The EML4-ALK MAF in cancer cells might be a predictive factor for crizotinib treatment. Chemotherapy was the optimal treatment method for this patient, who harbored EGFR T790M and C797G mutations in cis and had a synchronous EML4-ALK rearrangement.

Conflict of interest statement

No potential conflicts of interest are disclosed.

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