Clinical Case Report

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Responses to crizotinib and disease monitoring with circulating tumor cells in lung adenocarcinoma patient with MET exon 14 skipping mutation

A case report

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Abstract

Rationale: Mesenchymal-to-epithelial transition (MET) exon 14 skipping mutation was a targetable alteration in nonsmall-cell lung cancer (NSCLC), and the MET inhibitor of crizotinib had the most efficacy among all the targeted drugs. Most of the cancer-related deaths are associated with metastasis. Circulating tumor cells (CTCs) have been a valuable biomarker in assessing metastasis. Recent experiences suggested that CTCs detection may help improve diagnosis and predict prognosis for patients with NSCLC. However, few literatures have reported the CTCs detection based on the (MET) exon 14 skipping, which are positive in NSCLC patients.

Patient concerns: The patient, a 69-year-old Chinese male, with a 50 years history of smoking. Because of the cough, the patient went to the hospital and found the upper right lung tumor and the right supraclavicular lymph node enlarged. He was worried that it was cancer.

Diagnoses: The patient was performed biopsy of the right clavicle lymph node metastasis on October 12 and sent the tissue specimen for pathological evaluation. Finally, the patient was diagnosed to be with a pT3N3Mx stage IIIC lung adenocarcinoma.

Interventions: The patient began to take orally crizotinib 250 mg twice a day for the medical therapy after lymph node biopsy. At the same time, the CTCs were detected to observe the prognosis of the patients.

Outcomes: Compared with the first CTCs result, the second test revealed a decrease in the amount of CTCs, while the mesenchymal CTCs have increased, indicating the possibility of distal metastasis.

Lessons: This is the first proof that CTCs can be quantitatively assayed by MET exon 14 skipping mutation, which demonstrates the clinical response to crizotinib. More cases should be reported and further evaluation for treatment options and prognosis evaluation is necessary.

Abbreviations: CT = computed tomography, CTCs = circulating tumor cells, EMT = epithelial-mesenchymal transition, HGF = hepatocyte growth factor, MRI = magnetic resonance imaging, TKIs = tyrosine kinase inhibitors.

Keywords: exon 14 skipping, crizotinib, circulating tumor cells (CTCs), lung adenocarcinoma, MET

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

The authors have no conflicts of interest in this work.

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1. Introduction

Nowadays, lung cancer is one of the leading causes for global cancer-related mortality in both men and women, resulting in over 1 million deaths each year, while among all of the types, adenocarcinoma is the commonest histological one.[1] In the past 10 years, novel molecular targeted therapies were used by people for the treatment of lung adenocarcinomas.[2] MET proto-oncogene has long been the focus of therapeutic target in lung cancer. MET has played an important part in cell proliferation, apoptosis, and invasion, so does its ligand hepatocyte growth factor (HGF).[3,4] In MET, several alterations for Gain-in-functions have been achieved, such as gene amplification, protein overexpression, and mutations in the juxtamembrane and semaphorin domains.[5]

When mutations happen in MET exon 14 RNA splice acceptor and donor sites, it will lead to the skipping of exon 14 and the deletion of juxtamembrane domain,[6] and if the tyrosine 1003 in juxtamembrane domain is a binding site for CBL, an E3 ubiquitin ligase, ubiquitination, receptor endocytosis, and degradation will be caused in MET.[7] Therefore, exon 14 skipping is the mechanism for MET activation that shows elevated MET production, sketched-out signaling activation, and evident oncogenic capacity.[6] Now, in 4% of lung adenocarcinoma cases, MET exon 14 skipping has been used to report.[8] Preclinical systems support the use of MET tyrosine kinase inhibitors (TKIs) and crizotinibin tumors with MET exon 14 skipping,[8] and off-label use of crizotinib has also accumulated its clinical experiences in these patients. Recently, several case reports have already been using the MET exon 14 skipping to demonstrate the clinical responses to crizotinib in lung adenocarcinomas.[9–14]

Most cancer-related deaths are associated with metastasis. Metastasis is a multi-step process, while the blood stream is disseminated from primary tumors, and the CTCs will be present in the blood stream.[15] CTCs need to go through a process of epithelial-mesenchymal transition (EMT), through the loss of epithelial cells' characteristics and the acquisition of a mesenchymal phenotype, providing the cells with improved invasive potential for distal metastasis.[16] Evidence accumulated has implicated that CTCs can be used as a biomarker in the noninvasive monitor of cancer metastasis and offer information to help instruct therapy selection.[17] Because of their EMT markers, CTCs are a very heterogeneous population among the cells, which can be classified into 3 subpopulations, that is, epithelial CTCs, biophenotypic epithelial/mesenchymal CTCs, and mesenchymal CTCs,[18] and among all the 3 types above listed, mesenchymal CTCs are more easy to be found in the metastatic stages of cancer.[19]

Here, we reported a case of a patient with stage IIIC lung adenocarcinoma. In this case, the patient responded well to small-molecule MET inhibitor crizotinib with a novel MET exon 14 skipping mutation. And he died of brain metastases, which was consistent with the CTCs results, that though the total number of CTCs has decreased, the mesenchymal CTCs, indicating metastasis, increase.

2. Case report

The patient is a 69-year-old Chinese male, with a 50 years history of smoking. A computed tomography (CT) scan of the patient's chest on October 8, 2015, revealed a space-occupying mass measuring $13.2 \times 8.8 \times 9$ cm in size with multiple lymph node metastases (Fig. 1). After further evaluation of the serum tumor markers, such as CEA, CA125, CA15–3, CA19–9, ferritin, CYFRA21–1, WBC, SCCA, NSE, AST, and ALT, malignant pulmonary tumor is indicated in the patient's body. Then, physicians performed biopsy of the right clavicle lymph node metastasis on October 12 and sent the tissue specimen for pathological evaluation (data not shown). Finally, the patient was diagnosed to be with a pT3N3Mx stage IIIC lung adenocarcinoma.

In order to probe into the genomic profile of the tumor for targeted therapy, the tissue specimen of the patient's right clavicle lymph node metastasis and the related blood sample were sent for next-generation sequencing panel analysis after the consent was obtained from the patient and his family members. We have checked all of the genomic alteration types from the patient, including base substitutions, insertions and deletions, copy number alterations, and rearrangements on over 390 genes, which are normally connected with cancers. The genomic profile of the tumor disclosed 3 somatic mutations, including c.3025–3028+5del mutation of *MET* (NM_000245.2) gene, c.599–1G>A mutation of *PIK3R2* (NM_005027.3) gene, and Q38Kfs*6 mutation of *TP53* (NM_000546.5) gene. The MET c.3025–3028+5del mutation would have the consequence of 9 nucleotides deletion around the 3' splice donor site of MET exon 14, and result in exon 14 skipping (Fig. 2). MET exon 14 skipping

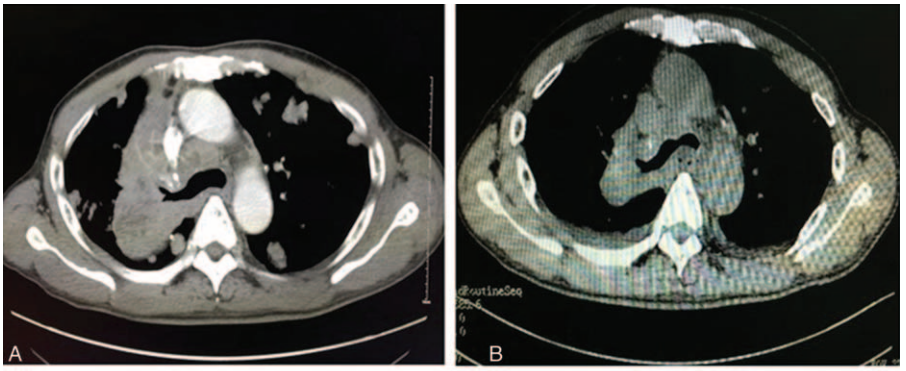

Figure 1. Response to crizotinib treatment. (A) Chest CT scans before crizotinib treatment showing a space-occupying mass with multiple lymph node metastases. (B) Chest CT scans after 4 weeks of crizotinib treatment showing a 42.4% reduction of the lung tumor, meeting RECIST partial response criteria.

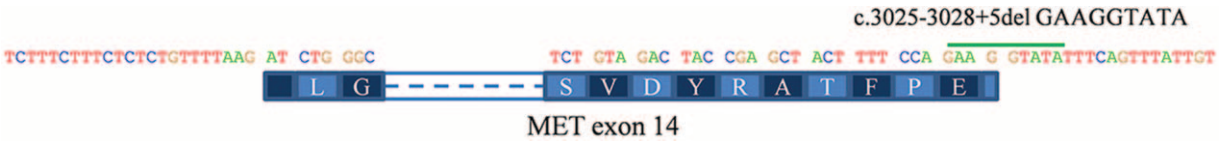

Figure 2. Diagram of MET c.3025–3028+5del mutation leading to exon 14 skipping.

has proved its function in mitigating MET degradation; in the meanwhile, it leads to the increasing of MET protein expression.[6] So, the patient began to take orally crizotinib 250 mg twice a day for the medical therapy since November 4, which has been provided off-label but with insurance validation. After 4 weeks of treatment, the CT scan of the patient on December 2 showed significant reduction of both lung tumor and lymph node metastases. His lung tumor measured $7.3 \times 5.3 \times 7.6$ cm in size, meeting RECIST partial response criteria (−42.4%; Fig. 1). Also, during the crizotinib treatment, the patient accepted the treatment with good tolerability and only experienced grade 2 diarrhea.

At the same time, in order to develop noninvasive liquid biopsy for distal metastases monitoring, 10 mL of peripheral blood of the patient was collected on October 13 for CTCs test with the agreement of the patient and his family members. CTCs test disclosed the existence of 7 epithelial CTCs and 3 biophenotypic epithelial/mesenchymal CTCs (Fig. 3). On December 4, CTCs test was implemented again, and 8 CTCs in total were confirmed to be 2 biophenotypic epithelial/mesenchymal CTCs and 6 mesenchymal CTCs (Fig. 3). Compared with the first CTCs result, the second test revealed a decrease in the amount of CTCs, while the mesenchymal CTCs have increased, indicating the possibility of distal metastasis. What is more, brain magnetic resonance imaging (MRI) scan, which the patient took, disclosed the multiple lesions in cerebrum, brainstem, and both cerebellar hemispheres, implicating brain metastases and consistent with the CTCs results. So, in the end, that patient died of respiratory failure caused by brain metastases 2 months later.

3. Discussion

Current report not only supports the idea that MET exon 14 skipping in lung adenocarcinoma is the oncogene but also shows that clinical responses can be quickly observed with the usage of MET inhibitor crizotinib. Unlike most splice site mutations that lead to the loss of reading frame and protein truncation, MET exon 14 splice site mutations will lead to the constitutive activity of MET. Commensurately, MET exon 14 skipping has been found and reported in 4% of lung adenocarcinomas, having the frequency as twice as that of MET amplification. Except above mentioned, MET exon 14 skipping has also been ascertained in the lung cancer of small cells,[20] glioblastoma multiforme,[21] and squamous cell head and neck cancers.[22] These data have proved the role of MET exon 14 skipping in the driving and impulsing of tumorigenesis, and it has also confirmed a distinct group of patients who are likely to gain benefit from MET inhibitors, such as crizotinib. As there are no clinical experiments currently that focus on the MET exon 14 skipping cancer population, the amassing of clinical responses recorded in case reports is the only form of clinical evidence revealing the targetability of MET exon 14 skipping. In the short run, it may emerge that cancer diseases with MET exon 14 skipping involved in will profit by anti-MET targeted treatment.

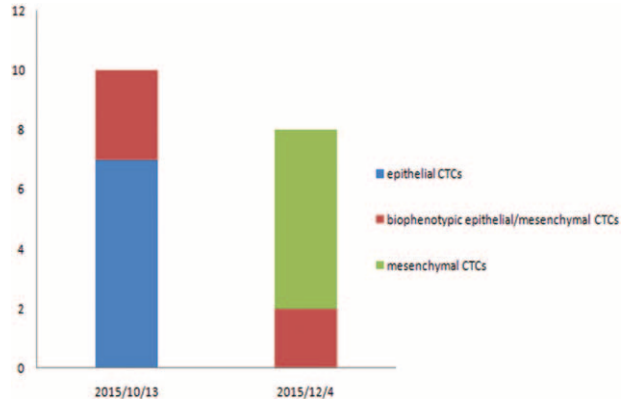

Figure 3. The number of single CTCs. CTCs test on October 13, before crizotinib treatment revealed 10 CTCs in total including none mesenchymal CTCs. CTCs test on December 4, after crizotinib treatment revealed 8 CTCs in total including 6 mesenchymal CTCs.

Crizotinib is a small molecule inhibitor of ALK, MET, and ROS1 receptor tyrosine kinase, which is potent and selective ATP-positive. The lung adenocarcinoma patient with MET exon 14 skipping mutation in this case has shown rapid clinical response to crizotinib treatment. For the reason that crizotinib's ability of penetrating the blood–brain barrier is quite lacked,[23,24] in the therapy of brain matastases, the function crizotinib can impose to the disease is very confined. That is why the patient in the case died for his brain metastases deteriorated despite the treatment's effects to the lung tumor.

Besides MET exon 14 mutation, genomic profile of the tumor has disclosed 2 other somatic mutations, c.599–1G>A mutation of PIK3R2 and Q38Kfs*6 mutation of TP53. *PIK3R2* gene encodes p85β, a managing subunit of PI3K that connects and stabilizes PTEN. c.599–1G>A mutation of PIK3R2 in this case is speculated to impair splicing, resulting in p85β mRNA degradation or protein abnormality, and the related results would hinder the interactions between p85 and PTEN; what is more, it would also promote PTEN degradation by ubiquitination. As a result, PIK3R2 mutation would lead to the activation of PI3K/AKT/mTOR signaling routes, which is included in cell growth, survival, proliferation, motility, and morphology; also, the preclinical study suggested that when the tumor cells are connected with PIK3R2 mutation, they would be sensitive to mTOR inhibitors.[25] Tumor suppressor p53, which is produced by *TP53* gene, will be either be lost or mutated in various malignancies.[26] No medicine targeting TP53 have been ever approved, although it was used to reveal that TP53 mutation was quite responsive to Wee-1 inhibitor MK-1775,[27,28] except that the clinical experiment NCT01357161, MK-1775, proved a preliminary response rate of 78.6% (11/14) in p53-mutant ovarian cancer. Thus, mTOR inhibitor everolimus, Wee-1 inhibitor MK-1775, or the unity of several targeted drugs may be used as an alternative for patients who have multiple mutations.

As mentioned above, CTCs can be used as biomarkers for noninvasive monitor cancer distal metastasis and mesenchymal CTCs is most closely associated with distal metastasis. Therefore, using EMT markers to differentiate CTCs helps to identify more aggressive CTC subpopulation and provides useful evidence for distal metastasis. In this case report, the increased mesenchymal CTCs were consistent with brain MRI results, which indicate the brain metastasis.

In summary, METexon 14 skipping mutation was a targetable alterative in NSCLC, and the MET inhibitor of crizotinib possesses the most efficacy among all the targeted drugs. As most of the cancer-related deaths are associated with metastasis, CTCs is a valuable biomarker in assessing metastasis. In this report, a patient case was analyzed and demonstrated to show that the one with MET exon 14 skipping mutation can respond to MET-directed targeted therapy, and CTCs can be used as biomarkers to noninvasive monitor cancer metastasis, as the patient did not appear to be unadapt to the treatment. As far as we know, this is the first report developing noninvasive CTCs for distal metastases monitoring in a lung adenocarcinoma patient with MET exon 14 skipping mutation that demonstrated clinical response to crizotinib. However, for the reason that the patient finally died of the respiratory failure caused by brain metastases, it is critically important that we develop the mechanism for the identification of new subsets of patients who will be likely to benefit from the targeted therapy as well as the noninvasive liquid biopsy for monitoring distal metastases to improve cancer patient care. We still have a long way to go in the exploring of that.

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