CASE REPORT

Molecular characteristics of multifocal invasive mucinous adenocarcinoma of the lung: Report of a rare case

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Keywords
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Abstract
Invasive mucinous adenocarcinoma (IMA) is an uncommon entity in the lung, with a poor prognosis. Multifocal IMA of the lung is even more unusual, and there is little experience with effective treatments. Herein, we present a case of multifocal IMA diagnosed in a 36 year-old man by video-assisted thoracoscopic surgery. A right middle lobe and a nodule in the right upper lobe were resected, as were mediastinal lymph nodes, leaving behind an autonomous right lower lobe nodule. To explore the feasibility of molecular treatment, next-generation sequencing of genetic mutations was performed after four cycles of chemotherapy (pemetrexed + cisplatin). Ultimately, a KIAA1468-RET fusion gene was detected at a disproportionate level (~67.3%), indicating that targeted therapy may be efficacious in treating this disease.

Introduction
Invasive mucinous adenocarcinoma (IMA) is uncommon in the lung, accounting for 2–10% of primary lung adenocarcinomas.¹ Compared to other variants of lung adenocarcinoma, cases of IMA carry a poorer prognosis.² A number of studies have linked IMA and KRAS mutations,³,⁴ and a lower prevalence of EGFR mutations and a higher prevalence of KRAS, ALK, and HER2 mutations were recently reported in conjunction with IMA, as opposed to other invasive adenocarcinomas.⁵ To the best of our knowledge, reports of an association between IMA and RET fusion genes are rare.

Case report
On May 19, 2015, a 36-year-old Chinese man was admitted complaining of dyspnea and chest discomfort that had lasted approximately two months, unaccompanied by hemoptysis or low-grade fever. No pertinent signs emerged during physical examination. The patient had generally enjoyed good health and had no history of smoking. All pulmonary function tests, as well as cranial magnetic resonance imaging, bone scan, and abdominal ultrasound, showed no obvious abnormalities. Serum levels of carcinoembryonic antigen, squamous cell carcinoma antigen, and neuron-specific enolase were also within normal reference ranges.

Subsequently, a CT scan revealed a sizeable mass (diameter 9 cm) within the middle lobe of the right lung. Its border at the upper lobe was poorly demarcated, and two small nodules in the right upper and lower lobes were also identified (Fig 1). A benign condition was considered, because the largest mass was ill defined and had not changed in size after two months of observation. Although fibrobronchoscopy was performed on two occasions, the bronchial lumen was devoid of lesions or external impingement, and no malignancy was identified in exfoliative cytology. Results of a positron emission tomography-computed tomography (PET-CT) scan were also compatible with a benign process of the right middle lobe (standardized update value 1.4). The nodules of the right upper and lower lobes failed to abnormally retain concentrated tracer, and no metastases were encountered in regional lymph nodes or in other areas of the body.
The middle lobe of the right lung and the small nodule in the right upper lobe were resected during video-assisted thoracoscopy on June 3, 2015. The mediastinal lymph nodes were also removed, leaving the separate lower lobe nodule intact. Upon examination, the growths were rich in mucus and highly gelatinous. Microscopic sections confirmed IMA at both the middle and upper lobe sites of involvement and in subcarinal lymph nodes (hematoxylin and eosin staining) (Fig 2a). Subsequent EGFR genetic testing (genes 18, 19, 20, and 21) was negative. The same was true of EML4-ALK genetic fusion testing.

Because the right lower lobe lesion remained unchanged after four chemotherapeutic cycles of pemetrexed/cisplatin (Fig 2b), the molecular structure of the tumor was explored. Profiling of genetic mutations via next-generation sequencing (OncoScreen TM 295 genes, Burning Rock Dx, Guangzhou, China) (Table S1) revealed a high proportion (~67.3%) of KIAA1468-RET fusion gene (Table S2). No other tumor-related somatic mutations were detected. The patient has survived for 20 months postoperatively, free of tumor recurrence or metastasis.

Discussion

The RET gene is a proto-oncogene located in the pericentromeric region of chromosome 10q11.2, which encodes a single-pass transmembrane, RTK. RET rearrangement is newly recognized as an oncogenic mutation in adenocarcinoma of the lung. The RET gene encodes a receptor tyrosine kinase that is critical in neural crest development, binding with its ligand (i.e. extracellular signaling molecules of the glial cell line-derived neurotropic factor family) to activate downstream pathways. Mutated RET genes are visible in a variety of cancers, such as hereditary medullary thyroid carcinoma of multiple endocrine neoplasia, but are seldom seen in normal lung tissue. To date, over seven RET fusion partner genes have been identified: KIF5B, the most common fusion partner gene with more than 10 variations; CCDC6; CUX1; TRIM33; NCOA4; KIAA1468; and KIAA1217. The RET tyrosine kinase in particular is preserved in all fusions and each RET partner protein contains a coiled-coil domain, which is believed to promote ligand-independent dimerization and constitutive activation of RET. Therefore, KIAA1468-RET, which was detected in our case, is preserved in the RET tyrosine kinase. This phenomenon has been documented in close succession by several sources, all conceding that the RET fusion gene is a new key virulence mutation in lung cancer. Cumulative data at present indicate that ~1.3% of patients with lung cancer harbor RET fusion genes, and nearly all corresponding tumors are adenocarcinomas. Although the current National Comprehensive Cancer Network guidelines (2014) clearly advise testing for RET fusion in patients with lung...
There are no targeted therapies directed specifically at RET as yet. Nevertheless, the following multi-targeted agents may variably inhibit RET kinase activity: vandetanib (inhibiting VEGFR-2/3, EGFR, and RET); sorafenib (inhibiting VEGFR-1/2, KIT, RET, CRAF, and mutated BRAF); sunitinib (inhibiting VEGFR-2, KIT, RET, and PDGFR alpha); and cabozantinib (inhibiting VEGFR-2, KIT, RET, MET, PLT-1/3/4, TIE-2 and AXL). No clinical trials for molecular subtypes of RET fusion have been completed, but research is ongoing.

Our patient was satisfied with the surgery undertaken (i.e. resected right middle lobe, upper lobe nodule, and mediastinal lymph nodes), which confirmed IMA in both lobes of the lung and in the subcarinal nodes (pathologic stage T4N2M0). However, the residual disease in the right lower lobe proved refractory to chemotherapy, with no prospect of further surgical removal, radiation, or other options.

It is well known that targeted therapy may significantly prolong survival in patients with adenocarcinoma of the lung. Given current diagnostic guidelines, most clinicians are inclined to test for EGFR and ALK only, despite the fact that there are few targeted therapies focusing on EGFR or ALK. The results of both EGFR and ALK testing were negative in our patient, but next-generation sequencing is capable of distinguishing more actionable genomic alterations than standard diagnostic methods. As genetic testing assumes greater importance in the clinical guidance of targeted therapy, the RET gene may increasingly become a potential target, enabling breakthrough treatments for patients with IMA of the lung.

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Disclosure
No authors report any conflict of interest.
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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Table S1 The detected 295 genes by next generation sequencing (OncoScreen TM 295 genes, Burning Rock Dx, Guangzhou, China).

Table S2 Next generation sequencing (OncoScreen TM 295 genes, Burning Rock Dx, Guangzhou, China): high proportion (~67.3%) of KIAA1468-RET fusion gene detected.