CASE REPORT

Dual drive coexistence of EML4-ALK and TPM3-ROS1 fusion in advanced lung adenocarcinoma

You-cai Zhu1, Xing-hui Liao2, Wen-xian Wang3, Chun-wei Xu4, Wu Zhuang5, Jian-guo Wei6 & Kai-qi Du1

1 Chest Disease Diagnosis and Treatment Center, Zhejiang Rongjun Hospital, Jiaxing, China
2 Tumor Molecular Laboratory, Zhejiang Rongjun Hospital, Jiaxing, China
3 Department of Chemotherapy, Zhejiang Cancer Hospital, Hangzhou, China
4 Department of Pathology, Fujian Cancer Hospital, Fujian Medical University Cancer Hospital, Fuzhou, China
5 Department of Medical Thoracic Oncology, Fujian Cancer Hospital, Fujian Medical University Cancer Hospital, Fuzhou, China
6 Department of Pathology, Shaoxing People’s Hospital, Shaoxing, China

Abstract

We report a case of concomitant EML4-ALK and TPM3-ROS1 fusion in non-small cell lung cancer (NSCLC) in a 47-year-old Chinese man and review the clinical characteristics of this type double of fusion. The patient presented with a local tumor of the left upper lobe and underwent thoracoscopy. Postoperative surgical pathologic staging revealed T1aN0M0 stage IA. Histological examination of the tumor showed lung adenocarcinoma. Ventana ALK (D5F3) assay of the left lung tissue was ALK negative; however, immunohistochemical assay was positive for ROS1 protein. Using next generation sequencing, we found that the tumor had concomitant EML4-ALK and TPM3-ROS1 fusion. No recurrence was observed during seven months of follow-up. Precise diagnostic techniques allow the detection of concomitant ROS1 fusion and other driver genes, including ALK or EGFR; therefore oncologists should consider this rare double mutation in NSCLC patients. Further exploration of treatment models is required to provide additional therapeutic options.

Introduction

Non-small-cell lung cancer (NSCLC) has the highest morbidity and mortality in the world. Incidence and mortality has risen in the female population and in developed countries. Two to 7% of NSCLC patients are ALK positive. Previous studies have indicated that ALK gene rearrangement is mutually exclusive to EGFR gene mutation in lung cancers. However, the coexistence of EML4-ALK fusion and EGFR mutations has occasionally been reported in a small proportion of patients at frequencies ranging from 0% to 8%. In addition, genomic rearrangements involving chromosomal rearrangements of ROS1 occur in 1–2% of NSCLC patients. A total of 15 ROS1 fusion partner genes have been reported in NSCLC, including CD74, SLC34A2, GOPC, CCDC6, SDC4, TPM3, EZR, LRIG3, KDELR2, LIMA1, MSN, CLTC, TPD52L1, FIG, TMEM106B, FAM135B, and SLC6A17. The clinico-pathological features of ROS1 fusions or ALK rearrangements are associated with a history of never smoking, younger age, and adenocarcinoma histologic type.
The gold standard for the detection of ALK rearrangements is break-apart fluorescence in situ hybridization. However, fluorescence in situ hybridization is usually only available in specialized institutions, while immunohistochemistry (IHC) is an easy, reliable, and cost-effective technique that is available in almost all pathological institutions. IHC can be used as an initial screening approach to assess ALK or ROS1 rearrangement in NSCLC. As a result of the progression of precise detection assays, such as next generation sequencing (NGS), rare cases of double positive in lung cancer have been reported.

To our knowledge, only two cases harboring ROS1 and ALK concomitant rearrangements have been reported in the literature. Herein, we report a third case of double TMP3-ROS1 and EML4-ALK rearranged NSCLC.

Case report
A 47-year-old Chinese male smoker (30 pack-year) presented to our hospital with a local tumor of the left upper lobe. He underwent thoracoscopy and lymph node dissection. The size of tumor was 2.0 × 1.8 × 1.2 cm (Fig 1). The postoperative surgical pathologic diagnosis was papillary predominant with visceral pleural involvement (T1N0M0 stage IA). Hematoxylin and eosin staining showed typical morphology of adenocarcinoma cells (Fig 2). IHC analysis was positive for TTF-1, CK7, and Napsin A, and negative for CK 5/6 and P63. Ventana ALK (D5F3) assay (Ventana Medical Systems, Inc., Roche, Tucson, AZ, USA) of the left lung tissue was ALK negative. However, IHC assay was positive for ROS1 protein (3+, 90%). We performed next generation sequencing (NGS) assay (Geneplus, Beijing, China) on the surgical specimen and found that the tumor had concomitant EML4-ALK fusion (abundance 0.92%) and TPM3-ROS1 (abundance 25.53%)

Discussion
The prevalence of a coexisting ROS1 gene rearrangement with other mutations is rare. Warth et al. screened 1478 completely resected NSCLCs with a ROS1-specific antibody and 68 cases (4.6%) showed ROS1 immunoreactivity. They demonstrated that ROS1 translocations occurred in conjunction with other driver mutations (EGFR, KRAS, and BRAF) but none harbored concomitant ROS1 translocation and ALK fusion. Wiesweg et al. detected ROS1 status using IHC in 805 patients with metastatic lung adenocarcinoma. Twenty-five lung cancer patients (4.8% of lung adenocarcinomas) showed ROS1-positivity and 36% of ROS1-IHC-positive cases presented with concomitant oncogenic driver mutations involving EGFR (6 cases), KRAS (2 cases), PIK3CA, and BRAF; however, ALK and ROS1 coexistence was not observed. Recently, Lin et al. conducted a study of 228 NSCLC patients with ROS1 rearrangement. Sixty-two cases were tested for ALK rearrangements, and EGFR and KRAS mutations, but none demonstrated concurrent ALK fusion or concurrent EGFR activating mutations. Lin et al. concluded that ROS1 rearrangements rarely overlap with alterations in other oncogenes. Therefore, concomitant ROS1 and ALK gene rearrangement is very rare. To date, only two cases harboring ROS1 and ALK concomitant rearrangements have been reported in the literature. Song et al. analyzed the data of 732 patients, 32 (4.4%) of which harbored ROS1 rearrangements. Of the 32 patients only one was identified with ALK/ROS1 coexistence. Uguen et al. reported a case of a 77-year-old female never-smoker
treated with crizotinib for three months who responded to this inhibitor; this was first case in a Chinese patient and only the second reported in the world.16

Crizotinib, an ALK/ROS1/MET inhibitor, was the first targeted agent approved by the United States Food and Drug Administration for the treatment of advanced ROS1-rearranged NSCLC based on a phase I crizotinib trial. It demonstrated an objective response rate of 72% and median progression-free survival of 19.2 months in advanced ROS1-rearranged NSCLC patients.20 Therefore, we hypothesized that advanced NSCLC patients with ALK/ROS1 coexistence may benefit from anti-ALK and anti-ROS1 treatment. However, as our patient is at stage IA, follow-up continues. Further reports of these rare cases are required to explore the best therapeutic strategy for advanced patients.

Immunohistochemistry has been demonstrated as a reliable prescreening test for detecting ALK expression in lung cancer in clinical practice.21 Currently, there are no approved companion assays for ROS1 fusion in NSCLC. Based on experience with ALK, commonly used methods to detect ROS1 fusion include FISH, IHC, reverse transcription-PCR, and NGS.22 However, IHC results may exhibit false positive because of aneuploidy leading to aberrant expression, as in our case, which demonstrated ALK protein negative by IHC but EML4-ALK fusion by NGS. Nowadays, in the era of personalized medicine, accurate multi-gene diagnostics is crucial. Developments in NGS have created a new method for the simultaneous detection of a large number of gene fusions.23,24 The incidence of double gene mutations in NSCLCs may be explained by different genetic alterations or multiple altered oncogenic pathways. In the future, NGS assay could be used to explore gene differential expression. Further studies are required to assess the performance of different NGS platforms.

In summary, cases of lung adenocarcinoma with concomitant ROS1 fusion and ALK gene rearrangement have been reported in advanced and early stage lung adenocarcinoma. IHC results may exhibit false positive because of aneuploidy, leading to aberrant expression. Currently, there is no consensus on standard therapy for tumors with double positive mutations or fusions. If concurrent driver mutations are identified, other testing assays should be considered to confirm the molecular diagnosis before proceeding with targeted therapy.

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Disclosure

No authors report any conflict of interest.

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