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CASE REPORT

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

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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 $T_{1a}N_0M_0$ 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.[1,2] Two to 7% of NSCLC patients are *ALK* positive.[3] Previous studies have indicated that *ALK* gene rearrangement is mutually exclusive to *EGFR* gene mutation in lung cancers.[4] 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%.[5,6] In addition, genomic rearrangements involving chromosomal rearrangements of *ROS1* occur in 1–2% of NSCLC patients.[7] 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*.[8–11] The clinicopathological features of *ROS1* fusions or *ALK* rearrangements are associated with a history of never smoking, younger age, and adenocarcinoma histologic type.[7,12]

The gold standard for the detection of *ALK* rearrangements is break-apart fluorescence in situ hybridization.[13] 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.[14] 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.[15,16] 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 ($T_{1a}N_0M_0$ 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%) (Fig 3). NGS assay was conducted using HiSEquation 4000 (Illumina Inc., Madison, WI, USA). After seven months of follow-up (to August 2017) no recurrence was observed.

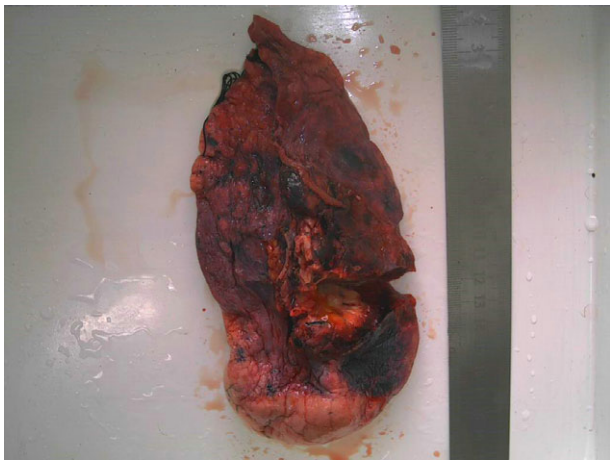

Figure 1 Gross pathologic findings; the mass size of the tumor was 2.0 cm × 1.8 cm × 1.2 cm.

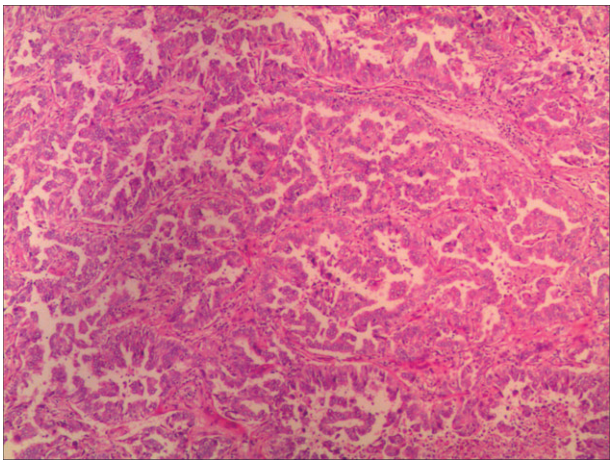

Figure 2 Hematoxylin and eosin staining of the left upper lobe revealed adenocarcinoma with a papillary growth pattern.

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.[17] 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.[18] 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.[19] 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.[15] 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

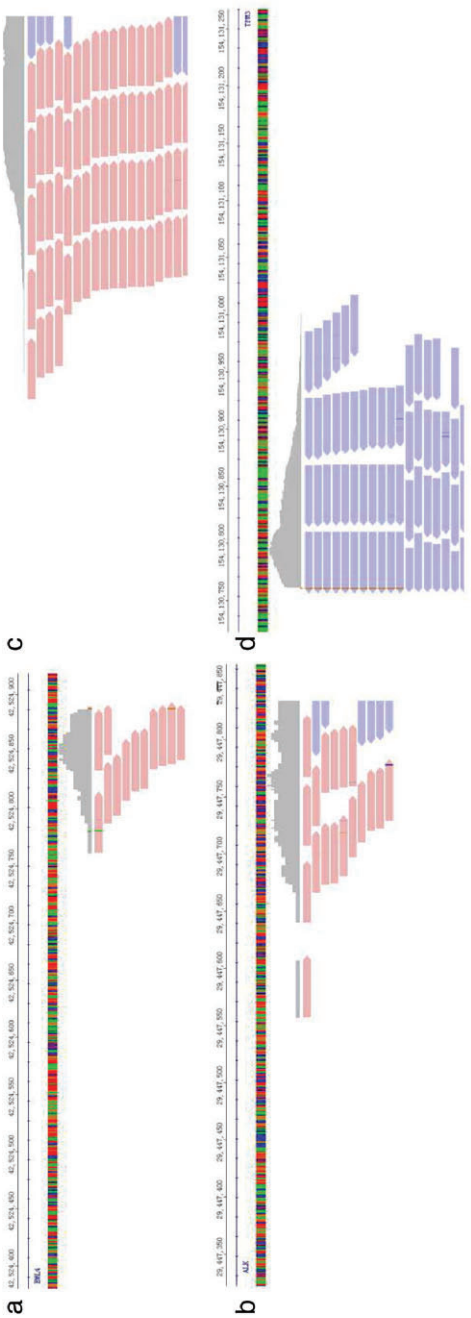

Figure 3 Paired-end sequencing data indicated somatic intrachromosomal (**a**) *EML4*, (**b**) *ALK*, (**c**) *TPM3*, and (**d**) *ROS1*, as demonstrated by the Integrative Genomics Viewer program.

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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