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Case Report

Different histopathology but the same clonality: ALK rearrangement in a patient with metastatic non-small-cell lung cancer

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Abstract: EML4-ALK rearrangement is detected in 2% to 7% of lung adenocarcinomas, these tumors are sensitive to crizotinib. The histologic feature of ALK translocated non-small-cell lung cancer (NSCLC) has been studied, presence of signet-ring cells was a powerful histologic indicator of ALK rearrangement, and this characteristic histology was present both in primary sites and metastases. However, the case we discribed here has different histomorphology in primary sites and metastases, but has the same genotype which both present ALK rearrangement, while absent of EGFR mutation, KRAS mutation and ROS1 rearrangement. This histologic heterogeneity may be a supplement of the histologic feature of ALK rearranged tumor. Moreover, genomic analysis can help distinguish clonal tumors from independent primaries.

Keywords: Lung adenocarcinoma, ALK rearrangement, metastasis, histology

Introduction

The EML4-ALK fusion oncogene represents a novel molecular target in non-small-cell lung cancers (NSCLC) [1]. The morphologic features of ALK+ were summarized by several studies, the presence of tumor cells with signet ring morphology was the most significant independent feature of ALK rearrangement in both primary lung adenocarcinomas and metastatic tumors [2-4]. These finding help pathologist identify cases that merit molecular testing, and find the suitable patients for targeted therapy. Furthermore, Lung cancer is a highly heterogeneous disease at the morphologic level. But it is rarely reported that there are NSCLC with different histomorphology between metastatic tumor and the primary tumor. In the current study, we describe a 36-year-old patient with metastatic NSCLC, We addressed the question whether the two foci with different histopathology are clonally related using molecular analysis.

Case report

Clinical history

A 36-year-old man who is a light-smoker (15 pack-year) was referred to local hospital in Feb 2013 because of a persistent non-productive cough and chest tightness for three days. A small lesion in the left low lobe of lung with pericardial effusion and enlargement of mediastinal lymph nodes were found on the chest computed tomographic scan (**Figure 1**). Two biopsy specimens was obtained by videoassisted thoracoscopic surgery: a tumor located at left lung lobe and the mediastinal lymph node.

Materials and methods

ALK immunohistochemistry

ALK IHC was performed on 4-μm sections of formalin-fixed paraffin-embedded (FFPE) tissues, using primary rabbit monoclonal anti-ALK antibody D5F3 (Cell Signaling Technology, Bill-

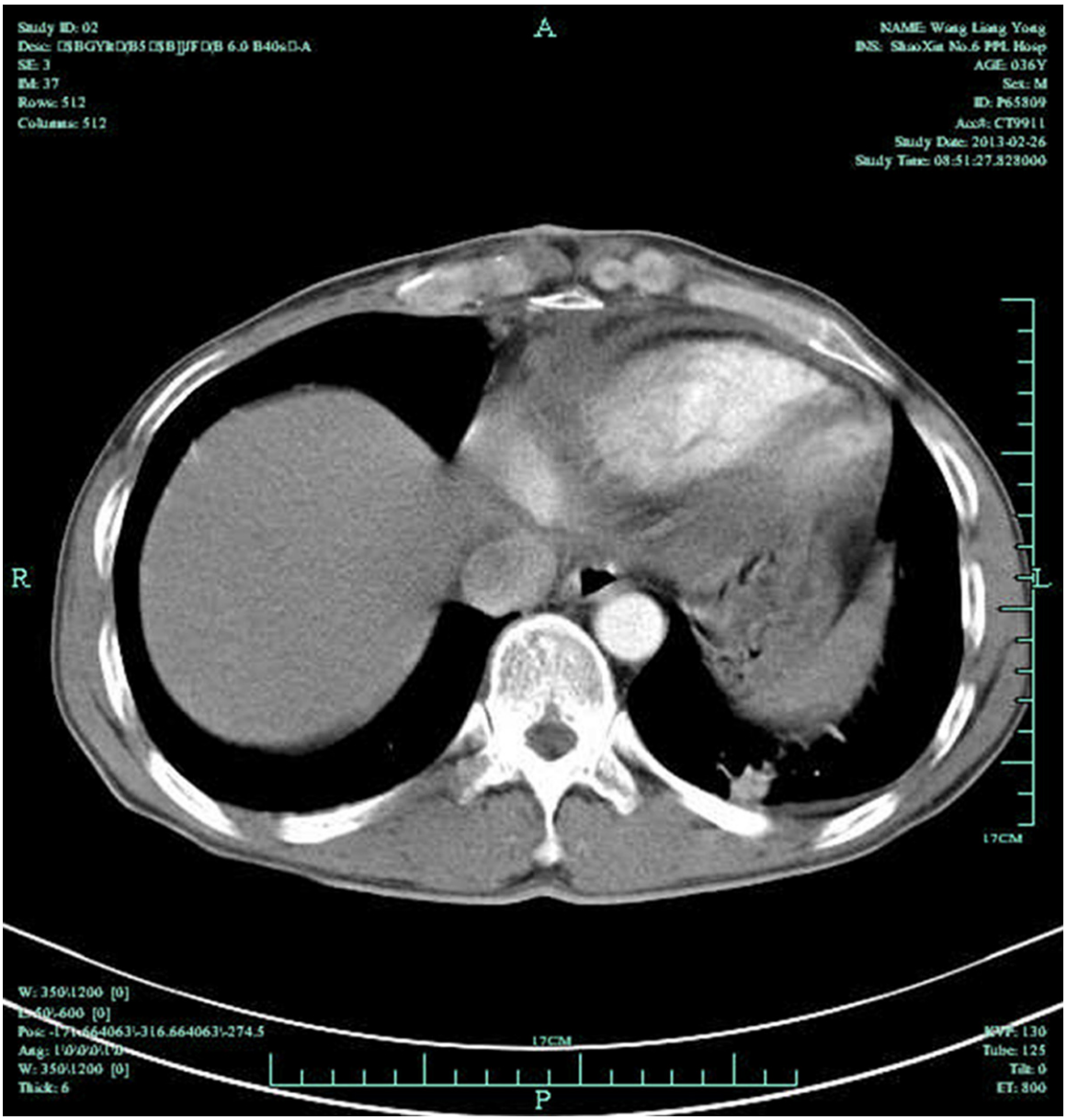

Figure 1. Chest radiograph and computed tomography revealed a primary lung mass and mediastinal lymph nodes.

erica, MA) with Dako EnVision detection kit, according to standard protocols [5].

ALK fluorescence in situ hybridization

The 4-μm-thick FFPE sections were used for evaluation of ALK genetic fusion status by FISH, using a break-apart probe to ALK (Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Molecular, Abbott Park, IL) according to the manufacturer's technical instructions and interpretation standard. Results were analyzed with a fluorescence Leica microscope and microsystem Imaging system (Leica Microsystems Inc., Buffalo Grove, IL).

Reverse transcription-polymerase chain reaction (RT-PCR) for ALK rearrangement and EGFR mutation

ALK rearrangement and EGFR mutation were performed with the ADx EML4-ALK Fusion Gene Diagnostic Kit and ADx EGFR Gene Mutations Fluorescence Polymerase Chain Reaction (PCR) Diagnostic Kit (Amoy Diagnostics Company Ltd., Xiamen, China) according to the

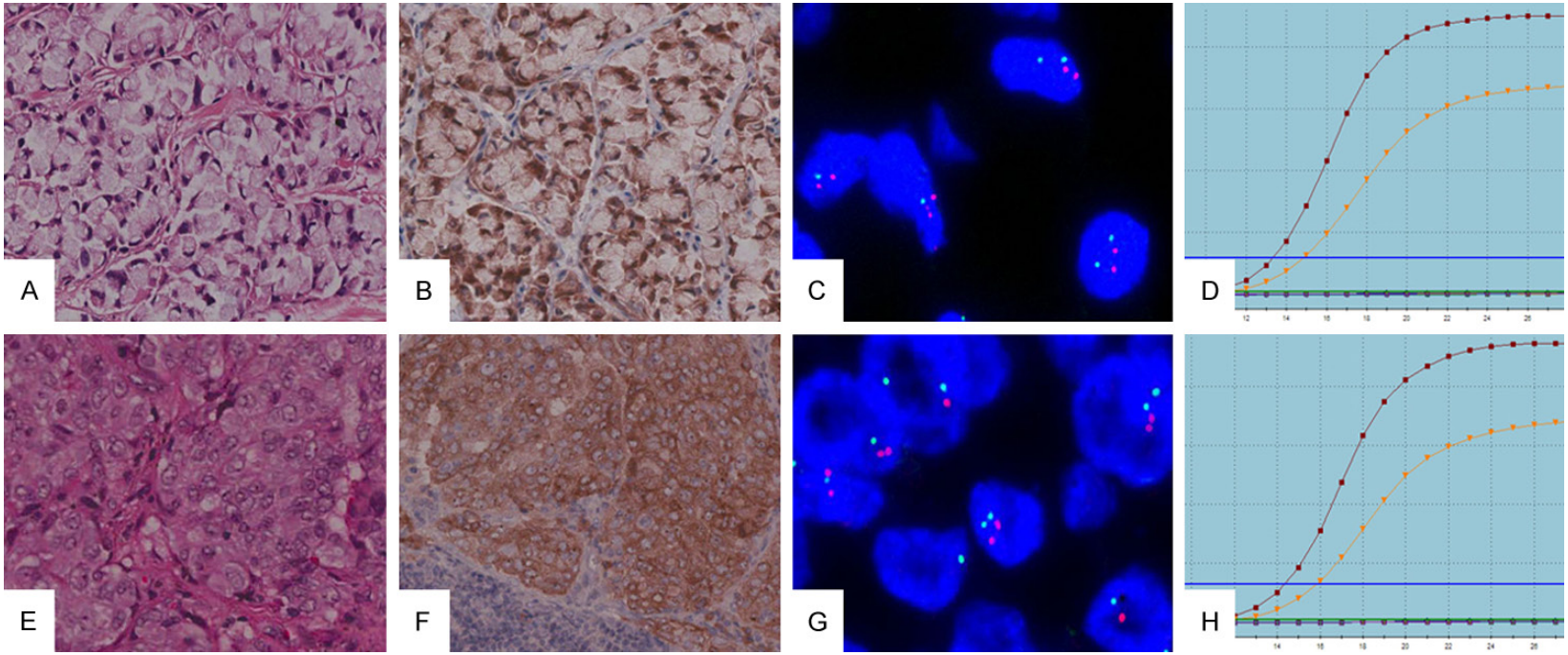

Figure 2. Hematoxylin and eosin (HE) staining, ALK immunohistochemistry, fluorescent in situ hybridization (FISH) and reverse transcription-polymerase chain reaction (RT-PCR) results for tumor in left lung lobe (upper panels) and the mediastinal lymph node (lower panels). Original magnification 200 × (A, B, E, F), 1000 × (C, G). (A) The lesion of left lung lobe reveal signet-ring adenocarcinoma in a solid growth pattern. (E) The lesion of lymph node presents hepatiod morphology, exhibiting abundant eosinophilic cytoplasm, round nuclei, and prominent nucleoli. (B and F) ALK immunohistochemistry showed diffuse cytoplasmic staining in both tumors. (C and G) FISH analysis revealed a split of red and green probes that flank the ALK translocation site in both tumor foci. (D and H) The translocations in both tumors were confirmed by RT-PCR.

manufactures' instructions on an Mx3000P instrument (Agilent Technologies, California, USA).

Results

Pathology finding of these two specimens were different obviously: the lesion of left lung lobe revealed signet-ring adenocarcinoma mostly in an solid growth pattern and partly in an acinar growth pattern, but the lesion of lymph node presented a solid growth pattern, with hepatiod tumor cells with abundant eosinophilic cytoplasm, round nuclei, and prominent nucleoli (**Figure 2**). This different morphology made us confused whether the patient had independent tumors or metastases. The result of IHC indicated their pulmonary origin: TTF-1 and CK7 staining were positive, while CDX2 and CK20 were negative in both tumors. What was more, strong positive staining of ALK (IHC Vantana) in both foci suggested that the patient was sensitive to crizotinib. At the same time, genomic analysis were carried out: ALK rearrangement were confirmed in both foci by two methods (RT-PCR and FISH), while EGFR, Kras, ROS1 were wildtype. The same immunologic and molecular phenotype demonstrated the metastasis and primary tumor may come from the same clonality.

Discussion

Tumor metastasis was the leading cause of death in lung cancer patients [6]. Metastasis was the process by which a tumor cell leaf the primary tumor, spreaded to a distant site via the circulatory system, and established a secondary tumor [7]. In general, Histomorphology of the metastasis was largely similar to that of the primary site, which leaded pathologists to find the nature of tumor. If some or all of the morphologic features were lost, it would be more challenging to diagnose using morphology-based methods. In our case, the primary tumor of lung was consisted of signet-ring cells, while the metastasis totally lacked signet-ring cells, solid pattern instead. The distinction was of great clinical importance as it influenced tumor staging and therapeutic strategy.

Up to date, this was the second case that ALK arranged NSCLC with different histomorphology. Yoshida found another case in which the primary tumor totally lacked signet-ring cells but the solid signet-ring cell pattern predominated in metastasis [3]. Would this distinct histomorphology is another feature of ALK-rearrangement lung cancer, further comparative molecular analysis of these distinct tumors would be of value to better understand the potential role of ALK in the pathogenesis of lung cancer.

We can get two hypothesis for these phenomenon: 1. metastatic tumor and the primary tumor were from the same clone, there were morphology change in the process of metastasis due to interactions between tumor cells and the host microenvironment [8]. 2. ALK rearrangement maybe a molecular change occurred in early stage of lung cancer. Some active tumor cells which had the primary molecular characteristics (ALK rearrangment) in primary site transfered into lymph node [9].

Moreover, ALK IHC played an important role in our diagnosis. The intense, granular cytoplasmic staining of ALK suggested us the possiblity of ALK arrangment in both lesions in the first time, which showed the right way for diagnosis and identifing the best therapy. As we and other researchers reported [5, 10], the concordance rate for ALK positivity between IHC and FISH is consistently high. Therefore, ALK IHC could prove to be a fast and cost-effective method as a screening tool before the FISH assay for the detection of ALK rearrangement.

Conclusion

We described a case of lung adenocarcinoma with different histological types between primary tumor and metastasis, but both haboring ALK-rearrangement conferring "sensitivity" to crizotinib. This case suggests that the morphology of metastasis in ALK-rearranged lung adenocarcinoma maybe very different from primary site, and molecular tools could provide a reliable and powerful way to evaluate the clonal relationships between multiple tumors.

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Disclosure of conflict of interest

None.

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