Changes in Immunohistochemical Protein Levels in Anaplastic Lymphoma Kinase-positive Lung Adenocarcinoma Possibly due to Chemo-radiotherapy

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Abstract

To detect the anaplastic lymphoma kinase (ALK) fusion gene in non-small cell lung cancer, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the standard methods. However, there are discrepancies between them. We herein report a 40-year-old woman with ALK fusion-positive adenocarcinoma that changed from positive to negative in IHC due to chemo-radiotherapy. Recurrence of the disease restored the IHC expression, whereas FISH was positive throughout the entire clinical course. Our experience suggests that we should therefore carefully evaluate samples after chemotherapy and radiotherapy.

Key words: ALK, IHC, FISH, lung, adenocarcinoma

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Introduction

Recently, the anaplastic lymphoma kinase (ALK) fusion gene, predominantly fused with the echinoderm microtubule-associated protein-like 4 (EML4) gene, has been identified as an important driver oncogene in non-small cell lung cancer. Although ALK-tyrosine kinase inhibitors such as crizotinib and alectinib are highly effective for ALK fusion gene-positive lung cancer, the identification of suitable recipients depends on a sensitive and specific screening test to detect ALK fusion. At present, three methods are available to detect ALK fusion: immunohistochemistry (IHC), fluorescence in situ hybridization (FISH) and reverse transcription-polymerase chain reaction (RT-PCR). IHC and FISH are the standard methods used in the clinical setting. However, several studies have reported false-positive and false-negative cases using IHC or FISH, as well as discrepancies between these methods. RT-PCR may be more sensitive and specific, but it requires multiple primer sets to detect all ALK fusion variants and high-quality RNA from samples. It is important to establish the accuracy of each diagnostic method in detecting ALK fusion and the cause of discrepancies between these methods. Therefore, we herein report a case of an adenocarcinoma with an ALK-positive status based on IHC, which changed to an ALK-negative status based on IHC following chemo-radiotherapy, suggesting that the protein levels determined by IHC change with treatment.

Case Report

A 40-year-old woman presented to our hospital with a non-productive cough. Chest radiography and computed tomography revealed a mass, 35 mm in diameter, in the right hilum. The patient had smoked 10 cigarettes per day for 12 years. A pathological diagnosis of lung adenocarcinoma was made following a bronchoscopic examination using endobronchial ultrasound-guided transbronchial needle aspiration. The disease was classified as stage IIIA (cT3N2M0) (UICC ver.7). The lung adenocarcinoma was negative for epidermal growth factor receptor mutations. Two months after the initial presentation, the patient received combined treatment of two cycles of cisplatin/vinorelbine with concurrent radiotherapy (60 Gy/30 Fr). Additional treatment was
given at the patient’s request: one cycle of cisplatin/pemetrexed/bevacizumab, followed by three cycles of pemetrexed/bevacizumab. This treatment greatly reduced the tumor and lymph node sizes, allowing surgical resection. Right upper lobe resection and bronchoplasty were carried out, seven months after the commencement of chemoradiotherapy and 50 days after the last cycle of chemotherapy. Complete resection was confirmed; however, a hypovascular tumor was detected in the left kidney 15 months after surgery. An ultrasonography-guided biopsy revealed a metastatic adenocarcinoma originating from the lung adenocarcinoma. The patient was thus diagnosed as having lung cancer recurrence.

As a novel ALK fusion gene had been identified, ALK fusion was examined using IHC and FISH for each of the following samples: sample A, a bronchoscopic biopsy of a lung tumor at the initial diagnosis prior to treatment; sample B, a surgical lung tumor specimen following chemo-radiotherapy; and sample C, an ultrasonography-guided biopsy of a renal tumor at the time of recurrence. A) IHC-positive (iScore 3), FISH-positive. B) IHC-negative (iScore 0), FISH-positive. C) IHC-positive (iScore 3), FISH-positive. The ALK IHC score (iScore) is assigned according to the percentage of IHC-positive tumor cells.

The examination using FISH showed that all samples were positive for the ALK fusion gene. However, the results of IHC differed; samples A and C (iScore 3) were positive, while sample B (iScore 0) was negative (Fig. 1). The ALK status of the tumor based on immunohistochemistry changed from positive to negative following chemo-radiotherapy, and changed back to positive at the time of recurrence. The patient was administered crizotinib, an ALK tyrosine kinase inhibitor. The tumor effectively decreased in size, and a partial response with a favorable performance status was thus reported at 26 months after the start of treatment (Fig. 2).

Discussion

The ALK fusion gene has recently been reported as the second driver mutation in non-small cell lung cancer (2). The fusion of ALK and EML4 occurs via a small inversion in chromosome 2. As result of this is that tyrosine kinase is constitutively activated, leading to tumor development and growth. The ALK gene is expressed in a few normal cell types, but the ALK fusion gene exists only in cancer cells, including lung cancer, malignant lymphoma, breast cancer, colon cancer and renal cell cancer (3, 4). As tyrosine kinase inhibitors, such as crizotinib and alectinib, are highly effective in patients with ALK-positive lung cancer (5), it is important to identify patients suitable for treatment with these inhibitors. IHC and FISH are routinely used to detect ALK fusion.

IHC using an anti-ALK antibody has been used to determine the tissue type in lymphoma. However, the ALK protein is not always detectable in lung cancer using this staining method, due to a low expression level (6, 7). Instead, an
antibody-enhanced polymer method was devised as a more sensitive approach. IHC is a relatively inexpensive method for detecting ALK fusion. However, the different partner fusion genes may influence the ALK levels detected using IHC. In contrast, FISH is a method that uses the hybridization of DNA probes labeled with a fluorescent dye to the ALK gene, which can be observed with a fluorescence microscope. FISH is an expensive method for detecting ALK fusion. It detects such fusion regardless of the different variants and partner genes. However, false-positive and false-negative results may sometimes occur given the short distance between the 3' and 5' ends of ALK.

In the present case, ALK fusion was detected using IHC and FISH at the initial diagnosis prior to treatment. The tumor was ALK fusion pseudo-negative based on IHC following chemo-radiotherapy, but it remained positive using FISH. The tumor was ALK fusion-positive using both IHC and FISH at the time of recurrence. If the protein levels are evaluated using IHC alone after chemo-radiotherapy, there is a possibility that the patient will be considered ALK-negative. Chemo-radiotherapy was highly effective in treating this tumor, and the decreased level of ALK protein expression may have been caused by the treatment. After interruption of the treatment, the cancer cells may have been re-activated, with a renewed expression of the ALK protein.

Several investigators have reported a similar phenomenon in cases of breast cancer. The expression levels of estrogen receptor, progesterone receptor, and HER2 receptor either decreased or were negative following chemotherapy (8). However, since the DNA remained in the cancer cells, positive results were obtained using FISH. In a study that examined the HER2 status in samples before and after neoadjuvant chemotherapy, the HER2 status changed from positive to negative in two cases following chemotherapy using IHC, although it remained positive using FISH (9).

To the best of our knowledge, no reports have described that the protein expression of the ALK fusion gene decreased after chemotherapy or radiotherapy. However, recent studies support the idea that IHC may not detect all cases with ALK fusion, as approximately 20-30% of FISH-positive cases were IHC-negative (10). There are several hypotheses for the discrepancy between IHC and FISH: the variability and activity of cancer cells; the type of variants and the partner fusion genes of ALK; the sample site, namely, primary or metastatic lesions; and technical artifacts, including late fixation or over-fixation.

It is important to detect ALK-positive cases because ALK tyrosine kinase inhibitors are highly effective treating for ALK-positive tumors. The sensitivity and specificity of IHC have been reported to be 67-100% and 93-100%, respectively (11, 12). IHC has a high negative predictive value of ≥98.9% (12). However, there is a potential for false negatives using IHC on post-chemotherapy or -radiotherapy samples. Our experience suggests that we should carefully evaluate samples after chemotherapy and radiotherapy. In addition, the ALK fusion status should be determined not only by IHC, but also by FISH, although this combination of diagnostic methods may be expensive.

The authors state that they have no Conflict of Interest (COI).

References


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