The Development Of A Highly Sensitive Mouse Monoclonal Antibody For Screening ALK Rearrangements In Lung Cancers

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Abstract

The anaplastic lymphoma kinase (ALK) rearrangements, mostly EML4-ALK fusion, occur in 3-7% of lung cancer patients and define a patient population that could respond to receptor tyrosine kinase inhibitor such as Crizotinib and Ceritinib [1]. Currently, ALK testing is mostly conducted by either fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) methods, each of which has its own limitations. The detection of the ALK protein in lung cancer patient tissues by IHC was found to be difficult mostly due to the low abundance of its fusion product. To overcome this limitation, we have developed an ALK mouse monoclonal antibody (clone 1A4) that is more sensitive than a current rabbit ALK antibody, clone D5F3. Our initial analyses revealed that 1A4 can correctly identify all 5 EML4-ALK positive samples that were previously confirmed by qPCR tests, while it did not yield significant background on any of the 30 EML4-ALK negative lung cancer samples. By using a different IHC detection system, we further tested 1A4 on 17 ALK-positive and 30 ALK-negative lung cancer biopsy specimens that have been validated by either FISH or PCR previously. The IHC result of the antibody agreed 100% with the previous molecular diagnosis. The high concordance of the IHC results by the novel ALK antibody with other DNA/RNA-based detection methods suggested that 1A4 could be used routinely for screening ALK rearrangements among patients.

Background

The percentage of tumor response, treatment duration, and smoking history. Selected tumor characteristics are listed in the table below the graph, with each table entry corresponding to a patient in the graph above [2].

Generating an ALK IHC antibody

Generating a high-affinity ALK antibody for IHC application. (A). The hybridomas screening process to select antibodies that recognize ALK fusion proteins in lung cancer cells [3]. (B). The ALK antibody, 1A4 clone, recognized full-length ALK protein that was transfected and expressed in 293T cells. (C). The ALK antibody, 1A4, recognized ALK fusion protein in lung cancer cell line [H2228] at the dilution of 1:110,000. (D). The IHC staining of ALK-positive anaplastic large cell lymphoma (ALCL) tissues and paraffin embedded xenograft tissues derived from H2228 cells.

Comparison between 1A4 and D5F3

The percentage of tumor response, treatment duration, and smoking history. Selected tumor characteristics are listed in the table below the graph, with each table entry corresponding to a patient in the graph above [2].

Analysis of IHC and ALK FISH results

The 1A4 staining result correlates with the ALK FISH and qPCR results in the lung adenocarcinoma sample set from Beijing Chaoyang Hospital. (A). Summary of the ALK diagnosis by different techniques. (B). Representative 1A4 staining of the ALK+ lung adenocarcinoma. (C). Representative 1A4 staining of the ALK-negative lung adenocarcinoma.

ALK antibody cocktails with higher sensitivity

The development of more sensitive ALK IHC antibodies by mixing several clones. The two-cocktail recipes were shown to have higher sensitivity than 1A4 did using a relatively insensitive detection system.

References