Incidentally Proven Pulmonary “ALKoma”

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Abstract

Genetic alterations of echinoderm microtubule-associated protein-like 4 (EML4)-anaplastic lymphoma kinase (ALK) inversion were recently found in lung cancer. A 39-year-old woman with multiple brain metastases and bulky mediastinal lymph node metastases was admitted. Biopsy from her supraclavicular lymph nodes was performed to differentiate the diagnosis between lymphoma and lung cancer. Pathologically, the lymph nodes had a feature of adenocarcinoma. On the other hand, the commercially available chromosomal fluorescent in situ hybridization (FISH) analysis showed split signals of ALK, which was confirmed to be the EML4-ALK inversion. The commercial-based ALK FISH is useful for screening pulmonary ALKoma.

Key words: EML4-ALK, lung cancer, oncogene addiction

(Inter Med 49: 603-606, 2010)
(DOI: 10.2169/internalmedicine.49.3126)

Introduction

The echinoderm microtubule-associated protein-like 4 (EML4)-anaplastic lymphoma kinase (ALK) inversion was recently detected in 6.7% of Japanese non-small cell lung cancer (NSCLC) patients (1). The fusion gene encodes a constitutive active oncoprotein with activated ALK kinase, resulting in the aberrant activation of the downstream signaling targets including Akt, signal transducer and activator of transcription (STAT) 3, and Ras-extracellular signal-regulated kinase (ERK) 1/2 (2).

The term ALKoma, coined by Benharroch et al, originally was used to represent anaplastic large cell lymphoma (ALCL) carrying the t(2 ; 5)(p23 ; q35) chromosome translocation (3). In 1994, Morris et al found that the t(2 ; 5) translocation fuses part of the nucleophosmin (NPM) gene on chromosome 5q35 to a portion of the ALK receptor tyrosine kinase gene on chromosome 2p23 (4). As with other fusion proteins found in hematological malignancies, ALKoma is also thought to become addicted to the ALK signaling pathway (3). Recently oncogene addiction has mainly been recognized among non-smoking NSCLC patients (5, 6). Just as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have become a mainstay of therapy for patients harboring EGFR mutation, patients with EML4-ALK inversion may benefit from therapy with ALK inhibitors. We herein report an incidentally proven EML4-ALK inversion in primary pulmonary adenocarcinoma.


Case Report

A 39-year-old woman was admitted to hospital because of generalized seizures. An initial screening head and body CT showed multiple brain metastases and swelling lymph nodes throughout the thorax (Fig. 1A, B). Fiber optic bronchoscopy showed direct invasion of tumor to the carina (Fig. 1C). After a crisis of generalized seizures, neurological disorders were not obvious. The patient’s performance status (PS) was graded as one, because of a dry cough, which had
The laboratory data, including the tumor markers (carcinoembryonic antigen and soluble interleukin 2 receptor) were normal. The white blood cell count was 11,770/μL, probably due to the prophylactic use of corticosteroids against seizures. As the patient was thought to be in need of immediate therapy, an open biopsy from her right supraclavicular lymph nodes under local anesthesia was performed on the day of the transfer. The frozen samples were subjected to pathological examination, to EGFR mutation analysis (the peptide nucleic acid-locked nucleic acid polymerase chain reaction (PCR) clamp method (7), Mitsubishi Chemical Medience, Tokyo, Japan), and to a comprehensive analysis for malignant lymphoma. Pathologically, the lymph nodes had a feature of moderately to poorly differentiated adenocarcinoma (Fig. 2A), with positive immunohistochemical staining for thyroid transcription factor-1 and epithelial markers (CAM5.2 and AE1/AE3). Immunohistochemical staining for lymphocyte markers was negative (CD20, CD45 RO, and CD30). Finally, her clinical diagnosis was determined to be cTxN3M1(BRA), clinical stage IV, adenocarcinoma of the lung. Although she was a young, never-smoking Japanese woman (8), she was found to be negative for EGFR mutations.

Meanwhile, the results of a comprehensive analysis for malignant lymphoma were reported. These analyses consisted of flow cytometric analyses with CD45 gating and a chromosomal G-banding analysis. In addition, the chromosomal fluorescent in situ hybridization (FISH) analyses were performed to detect the transition of ALK (2p23), BCL6 (3q27), IGH/BCL1 t(11; 14)(q13 ; q32), IGH/BCL2 t(14 ; 18)(q32 ; q21), and IGH/CMYC t(8 ; 14)(q24 ; q32), based on a pathologist’s decision (“ML-NET”, SRL, Tokyo). In this case, the FISH analyses were added because the sample was not adequate for G-banding. Surprisingly, the FISH analysis of ALK, using 5’-(green) and 3’-(red) sequences for hybridization probes, showed the split signals of ALK, in up to 96% of the cells counted (total 100 cells) (Fig. 2B). In order to analyze the counterpart of transition for ALK, multiplex reverse transcription PCR of the EML4-ALK fusion transcripts was performed by YLC, MS and HM, and the transition was found to be EML4-ALK inversion, variant 2 (Fig. 2C).

Abbreviations: PCR: polymerase chain reaction, FISH: fluorescent in situ hybridization

Discussion

EML4-ALK inversion was first identified by Soda et al, from a lung adenocarcinoma specimen that was surgically resected from a 62-year-old male with a history of smoking. They made a cDNA library from the specimen, inserted cDNAs into the plasmid clones, and then infected them into mouse 3T3 fibroblasts with recombinant retrovirus to assess its ability to transform the foci. The EML4-ALK inversion transcripts were found in one of the transformed foci (1).

ALK, as well as leukocyte tyrosine kinase (LTK), is a re-
Figure 2. Histopathology and genetic analyses. Moderately to poorly differentiated adenocarcinoma is recognized with acinar patterns (A). A genomic FISH analysis showed that 96% of the cells which were analyzed had the split signal of ALK. A representative cell is shown (B). Multiplex RT-PCR to capture all in-frame fusions between \( EML4 \) and \( ALK \) messages was conducted with the following primers; 

- 5'-GTGCAGTGGTAGGACATTGGGG-3',
- 5'-AGCTACATCACACCATGGACTGG-3',
- 5'-TACCAAGTGCTGTCAAATTGACGG-3',
- 5'-GCTTTCCCCGCAAGATGGACGG-3',
- 5'-CAGCTGAGAGAGTGAAAGCTTTGG-3',
- 5'-GACAGTTGGAGGAATCTGTCGATG-3',
- 5'-ATCCTGCGGAACACTATTCAGTGG-3',
- 5'-TCAAGCACATCTCAAGAGCAAGTG-3',
- 5'-TCTTGCCAGCAAAGCAGTAGTTGG-3'.

Examination of an enlarged lymph node revealed the successful amplification for the \( EML4-ALK \) variant 2 transcript (indicated as #J4).

Receptor tyrosine kinase similar to the insulin receptor subfamily of kinases. \( LTK \) is found in murine B lymphocyte precursors and in forebrain neurons. \( ALK \) is usually found in the nervous system, where it serves the normal neural differentiation and construction. By transfusing its kinase domain with an activating counterpart with coiled-coil domain, like \( NPM, \) TRK-fused gene (\( TFG \)), \( EML4 \) and so on, \( ALK \) gains oncogenic potential via a constitutional dimerization (2).

Since the receptor tyrosine kinases are one of the main targets of therapy in malignancy, Rikova et al performed a global survey of phosphotyrosine using lung cancer cell lines and clinical samples (9). Along with the well-known phosphorylation of EGFR and MET, the tyrosine phosphorylation of ALK was found in one cell line and in seven patients. A further analysis revealed three \( EML4-ALK \) inversions and one \( TFG-ALK \) fusion in 103 NSCLC patients, thus resulting in an overall frequency of ALK fusion of 4% in the Chinese population (9). A NSCLC cell line, H3122, which harbored an \( EML4-ALK \) inversion, showed massive apoptosis with an ALK kinase inhibitor, TAE-684 (10). Furthermore, transgenic mice expressing \( EML4-ALK \) conditionally in lung alveolar epithelial cells, which developed innumerable lung adenocarcinomas within a few weeks after birth, responded greatly with the oral administration of small-molecule inhibitors of the ALK kinase (11). Therefore, ALK is a novel therapeutic target in NSCLC. A phase I trial using PF02341066, TKI for MET and ALK, is ongoing for NPM-ALK-positive lymphoma (NCT00585195).

The clinicopathological background in patients with \( EML4-ALK \) inversion has been previously well described in two series. Inamura et al described that \( EML4-ALK \) positive lung cancers are characterized by an acinar histology, harboring neither \( EGFR \) mutation nor \( KRAS \) mutation, a non- or light smoking background and a young onset (12). Another group reported similar findings in which patients with \( EML4-ALK \) inversion were younger, more likely to be never/ light smokers. They have also shown mutations of \( KRAS \), \( EGFR \) and the rearrangement of \( EML4-ALK \) to be mutually exclusive (13). Furthermore, the latter group focused on the higher incidence of metastatic diseases in patients with an \( EML4-ALK \) inversion or \( EGFR \) mutation compared to patients without those alterations. The present case closely matches these findings. It is therefore suggested that an ALK FISH analysis should be recommended in the case of a never/light smoker, of younger onset, who is negative for \( EGFR \) mutation and advanced diseases.

Although several counterparts of \( ALK \) transition have been reported in some kind of tumors (2), the \( EML4-ALK \) inversion occurs most frequently in NSCLC (14). The identification of fusion transcripts is somewhat difficult because of the variation in the breakpoints of inversion. Soda et al are in the process of establishing multiplex RT-PCR for detecting fusion transcripts (1, 15). However, taking into consideration that there are a few other types of \( ALK \)-fusion (\( TFG \) and \( KIF5B \) (16)), the commercially used, previously established ALK FISH analysis is more useful in the screening of \( ALK \) altered NSCLC.

Since the incidence of patients demonstrating NSCLC with ALK transition who may benefit from a timely diagnosis and appropriate therapy exceeds the incidence of ALCL, the commercially-based ALK FISH is therefore considered to be a promising diagnostic modality for determining NSCLC patients with \( ALK \) transition.

Abbreviations: TKI: tyrosine kinase inhibitor, TRK-fused gene

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Inter Med 49: 603-606, 2010 DOI: 10.2169/internalmedicine.49.3126
Acknowledgement

We thank Brian Quinn for critical review and Yoko Takeda for her valuable technical assistance.

References