A case of lung adenocarcinoma with a concurrent EGFR mutation and ALK rearrangement: A case report and literature review

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Abstract. The echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase (EML4-ALK) fusion is almost mutually exclusive to epidermal growth factor receptor (EGFR) or K-RAS mutation in non-small cell lung cancer (NSCLC), and it is extremely rare for patients to exhibit both mutations. The present study reported the case of a 71-year-old female diagnosed with adenocarcinoma, exhibiting mutations in EGFR and EML4-ALK. The present study treated this patient with EGFR-TK inhibitors, as the first line therapy, and gefitinib therapy revealed a good response until now. In addition, previously reported cases and associated literature were reviewed. The present study provided a greater understanding of the molecular biology and optimal treatment for patients with NSCLC with >1 driver mutation.

Introduction

Lung cancer is one of the most common types of malignant tumor worldwide, of which non-small cell lung cancer (NSCLC) accounts for >85%. The trend of lung cancer treatment has been towards personalized therapy and molecular targeted therapy is currently one of the most popular and promising fields of advanced NSCLC treatment. Driver genes, including epidermal growth factor receptor (EGFR) and echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase (EML4-ALK) are common target genes, and the success of the clinical application of inhibitors against these two molecular targets have been demonstrated in East Asia (1,2). Previous studies suggested that the EML4-ALK fusion is almost mutually exclusive to EGFR or K-RAS mutation in NSCLC (3-5). However, at least 11 patients exhibiting both an EGFR mutation and the EML4-ALK fusion have been reported worldwide (6-13). The present study reports a twelfth case and also the first case of a patient of northern Han Chinese ethnicity who exhibits the two concomitant mutations. The most effective treatment for these two gene-positive patients remains to be elucidated with discordant results reported previously in the literature. All protocols in the present study were approved by the Human Clinical and Research Ethics Committees of the First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China) and the General Military Hospital of Beijing PLA (Beijing, China). The patient provided written informed consent.

Case report

A 71 year-old female who has never smoked and originates from Tongchuan City (Shan'xi, China), was first admitted to The First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China) as a result of left leg pain and swelling, without fever, in March 2013. Imaging examination, including computed tomography (CT) and magnetic resonance imaging (MRI), revealed a 40 mm tumor in her left lower lung (Fig. 1A) and left tibia destruction with a soft tissue mass (Fig. 2A). No significant previous medical history and laboratory findings were reported. The surgery on the left tibia was performed with the pathological diagnosis of poorly differentiated metastatic adenocarcinoma of the tibia from the left lower lung, clinical stage IV (cT3N3M1b; Fig. 3A and B). Following diagnosis, the patient was referred to The General Military Hospital of Beijing PLA (Beijing, China) in April 2013. A further lung biopsy was not performed considering the patient's clinical status. Gene detection for mutations in EGFR (Fig. 4A) and EML4-ALK (Fig. 4B) was performed on a formalin-fixed, paraffin-embedded tibia tumor specimen, by reverse transcription-quantitative

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Key words: non-small cell lung carcinoma, epidermal growth factor receptor gene mutation, echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase fusion gene, adenocarcinoma, gefitinib

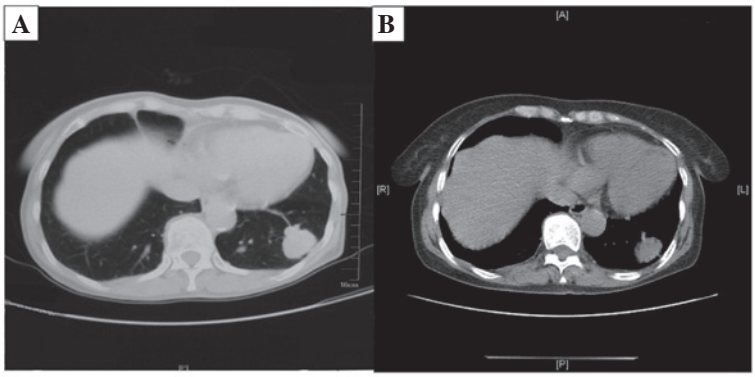

Figure 1. Initial CT scan of the thorax and following treatment with gefitinib. CT scans were captured in the First Affiliated Hospital of Xi'an Jiaotong University (A) prior to treatment and in the General Military Hospital of Beijing PLA (B) 186 days after treatment with gefitinib. CT, computed tomography.

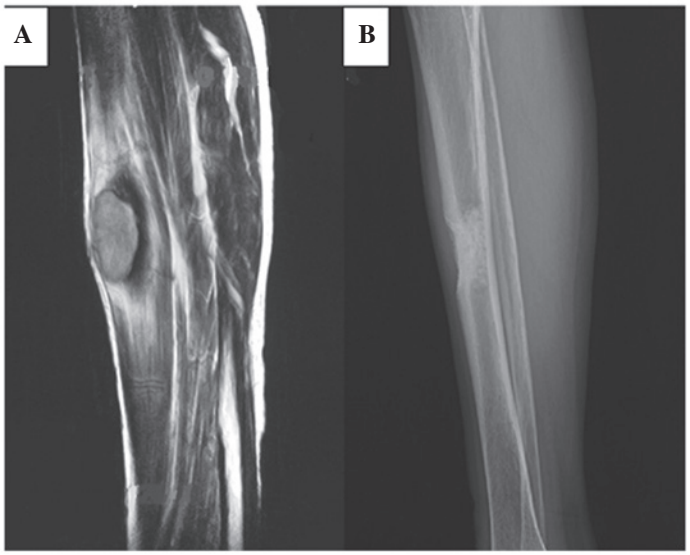

Figure 2. Initial MRI scan of the leg and following treatment with gefitinib. MRI scans were captured in the First Affiliated Hospital of Xi'an Jiaotong University (A) prior to and an X-ray plain film was captured at the General Military Hospital of Beijing PLA (B) following after treatment with gefitinib. MRI, magnetic resonance imaging.

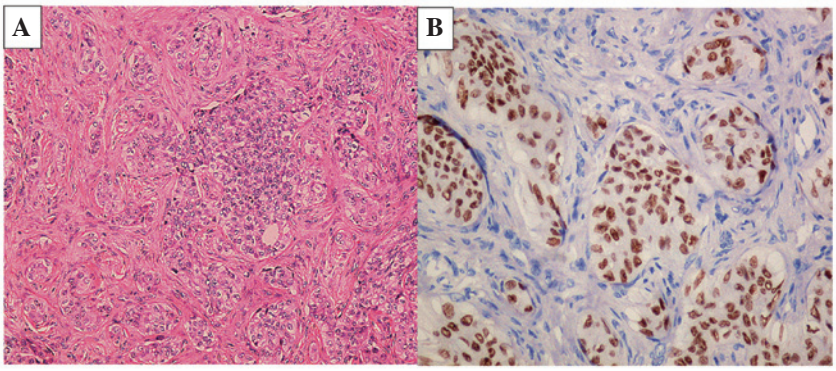

Figure 3. Histology of the bone metastasis. (A) The hematoxylin-eosin staining revealed that tumor cells were transformed lung adenocarcinoma cells (magnification, x200). (B) Immunohistochenmical examination revealed that the tumor cells were positive for staining with the monoclonal anti-TTF-1 antibody (magnification, x200).

polymerase chain reaction. The genomic DNA was extracted using QIAamp DNA FFPE Tissue kit (Qiagen, Inc., Hilden, Germany) and the total RNA was extracted using an RNeasy Mini kit (Qiagen, Inc.), and was reverse-transcribed into cDNA using RevertAid™ First Strand cDNA Synthesis kit (Fermentas, Thermo Scientific, Wilmington, DE, USA). The human EGFR mutation qualitative detection kit was the EGFR ADx-ARMS kit and the human EML4-ALK gene expression

Table I. Clinical features of 12 patients with the EML4-ALK fusion gene and *EGFR* mutation.

Patient, ref	Ethnicity	Age/gender	Smoking history	Histology	Primary lesion	TNM stage	*EGFR* mutation status	EML4 ALK	First line treatment	Assessment
1, 6	Chinese	44, F	Never smoked	Adenocarcinoma	Left upper lung lobe	$cT_{2a}N_3M_{1b}$ (left ribs and thoracic vertebral bodies) stage IV	Exon 19 deletion	Variant 6	Gefitinib	Partial response (122 days following treatment)
2, 6	Chinese	56, F	Never smoked	Adenocarcinoma	Right upper lung lobe	$cT_4N_3M_{1b}$ (brain) stage IV	Exon 21 L858	Variant 1	Gefitinib	Partial response (36 days following treatment)
3, 6	Chinese	50, M	45 pack-years	Adenocarcinoma	Right upper lung lobe	$cT_3N_0M_{1a}$ (pleura) stage IV	Exon 21 L858	Variant 1	Sequential gemcitabine/ carboplatin+erlotinib	Partial response (8 weeks following treatment)
4, 6	Chinese	70, M	Never smoked	Adenocarcinoma	Right upper lung lobe	$cT_1bN_3M_{1b}$ (brain) stage IV	Exon 21 L858	Variant 1	Erlotinib	Partial response (105 days following treatment)
5, 7	Chinese	56, F	Never smoked	Adenocarcinoma	Right upper lung lobe	$T_1N_0M_0$ stage Ia	Exon 19 deletion	Variant 1	Surgery	R_0 resection[a]
6, 8	Chinese	72, F	Never smoked	Adenocarcinoma	Right upper lung lobe	Stage IV (brain bone metastasis)	Exon 19 deletion	Variant 1	Gefitinib	Partial response (232 days following treatment)
7, 9	Caucasian	48, M	Never smoked	Adeno-squamous carcinoma	Right upper lung lobe	$cT_1N_0M_1$ (left tenth rib)	Exon 19 deletion	-	Cisplatin + gemcitabine six cycles	Partial response (2 months following treatment)
8, 10	Caucasian	65, F	Never smoked	Adenocarcinoma	Right upper lung lobe and right hilar	$pT_2N_2M_0$ stage IIIA	Exon 19 deletion	-	Erlotinib	Complete response (25 months following treatment)
9,11	Japanese	39, M	Light smoker	Adenocarcinoma	Right upper lung lobe	$cT_4N_3M_{1b}$ (bone) Stage IV	Exon 21 L858	Variant 1	Cisplatin + docetaxel three cycles	No clinical benefit
10, 12	Caucasian	52, F	Heavy smoker	Adenocarcinoma	Left upper lung lobe	$cT_{1b}N_2M_0$ stage IIIa	Exon 19 deletion	-	Chemotherapy+ gefitinib+ radiotherapy	Stable disease (7 months following treatment)
11,13	Chinese	56, M	Heavy smoker	Adenocarcinoma	Right upper lung lobe	$T_4N_2M_{1a}$ stage IV	Exon 19 deletion	-	Gemcitabine/cisplatin+ erlotinib+radiotherapy +crizotinib	Complete metabolic response (24 months following treatment)
12, present study	Chinese	71, F	Never smoked	Adenocarcinoma	Left hilar	$cT_3N_3M_{1b}$ (bone) stage IV	Exon 19 deletion	Variant 1	Gefitinib+erlotinib	Stable disease (7 months following treatment)

The case presented by Koivunen *et al* (5) reported mentioned no patient information, therefore, this has not been shown in the table. [a]Complete resection with no microscopic residual tumor. '-' indicates that the EML4-ALK fusion gene was positive, but the variant was not reported. EML4-ALK, echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor.

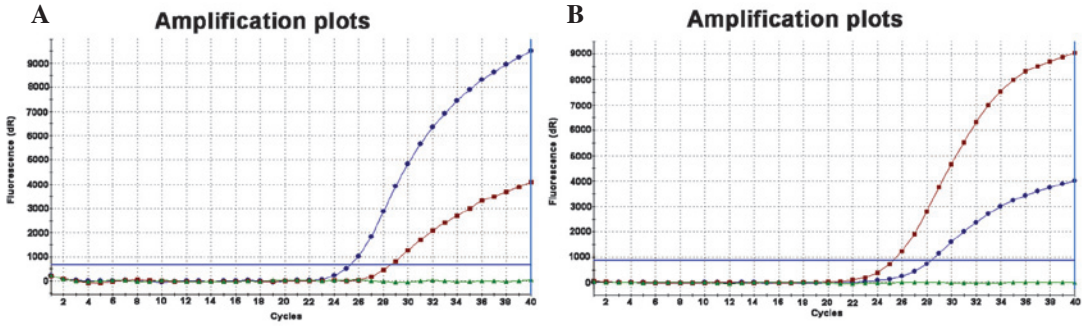

Figure 4. (A) EGFR19 exon amplification curve and (B) EML4-ALK amplification curve results. The blue, red and green represent the sample, positive control and negative control, respectively. EML4-ALK, echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor.

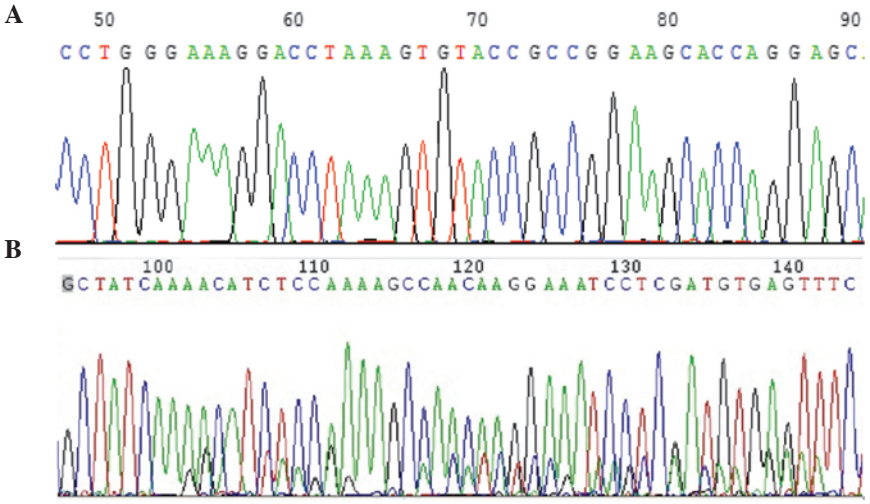

Figure 5. (A) Variant 1 of EML4-ALK translocation in bone metastasis. (B) Deletion in EGFR exon 19 (heterozygous 2235_2249del15; E746_A750del) in bone metastasis. EML4-ALK, echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor.

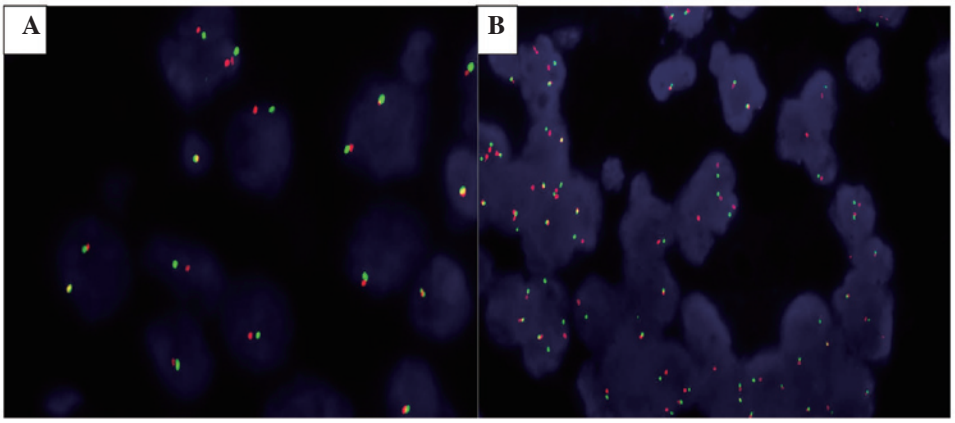

Figure 6. Fluorescent *in situ* hybridization reveals a split of red and green probes, which flank the ALK translocation site in an EML4-ALK-positive tumor. (A) Large nucleus sections; (B) small nucleus sections. Staining with 4',6-diamidino-2-phenylindole; magnification, x1,000. EML4-ALK, echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase.

assay kit was the EML4-ALK ADx-ARMS kit (Amoy ADx Ltd., Xiamen, China). The amplification products were sent to Beijing Jin Weizhi Biological Technology Co., Ltd. (Beijing, China) for sequencing and a variant 1 of the EML4-ALK translocation (Fig. 5A) and an EGFR exon 19 deletion 2235-2249 (delE746-A750) were detected (Fig. 5B). An ALK rearrangement was further confirmed by fluorescence *in situ* hybridization using the commercially available breakapart probe (Guangzhou LBP Medicine Science & Technology Co., Ltd., Guangzhou, China; Fig. 6). The patient was administered 250 mg gefitinib orally every day as the first line chemotherapy and linear accelerator radiotherapy at the left

tibia by PTV 2 Gy/30 Gy/15F/23 days. Following 5 months of EGFR-tyrosine kinase inhibitor (TKI) therapy and 15 radiotherapy sessions, imaging examination demonstrated that the left middle tibia cortex was absent, and the bone mineral density was increased (Fig. 2B). Gefitinib therapy revealed an acceptable response with a repeated chest CT (Fig. 1B), which demonstrated no obviously enlarged lesions or novel lesions following 8 months of therapy.

Discussion

The *EGFR* gene, located on the 12-14 region of the short arm of chromosome 7, consists of 28 exons and the majority of mutations were located within exons 19-21 of the tyrosine kinase (TK) domain (14-16). Deletions in exon 19, with the highest mutation rate among the total *EGFR* mutation, are associated with increased gene expression and TK inhibitor sensitivity (14,17). The EML4-ALK fusion gene was first identified by Soda *et al* in 2007 (3) in a Japanese patient with NSCLC, which is formed by a small inversion within chromosome 2p, and at least 11 different variants have been previously identified, with variants 1 and 3 being the most common (3-6,16,18-23).

EGFR-TK inhibitors, gefitinib and erlotinib, have been widely used for the treatment of patients with advanced NSCLC exhibiting the *EGFR* mutation. Markedly improved benefits were observed from EGFR-TK inhibitors in a previous study, which compared the efficacy of conventional cytotoxic chemotherapy as first-line treatment (24). In the 2012 edition of the NCCN clinical practice guidelines of NSCLC, *EGFR* mutation detection was suggested in the initial treatment of patients with advanced NSCLC (25). Patients who harbor the EML4-ALK mutation fail to benefit from EGFR-TKIs (5,21). Crizotinib, an orally bioavailable ALK inhibitor, is recommended for treating these patients (26) and is currently under phase III clinical trials worldwide. Whether EML4-ALK NSCLC can behave in an analogous manner to the *EGFR* mutant NSCLC and whether crizotinib can become a milestone in the treatment of NSCLC remains to be elucidated.

Patients who exhibit both mutations are extremely rare and previous studies have suggested that an EGFR mutation and EML4-ALK gene fusion are mutually exclusive molecular events (3-6,18,20-22,27). The literature for both the *EGFR* and EML4-ALK mutations in NSCLC was assessed and revealed only 12 cases, including the present case (Table I). This is also the first case, to the best of our knowledge, in the northern Han Chinese population identified with a concurrent *EGFR* exon 19 deletion 2235-2249 (delE746-A750) and EML4-ALK variant 1. As shown in Table I, the clinical characteristics of the 12 cases of patients with advanced stage NSCLC with the concomitant mutations presented in our study were as follows: Median age, 57; 7/12 female; 9/12 Asian (8/12 Chinese, 1/12 Japanese); 9/12 light-smoker or never-smoked; pathological type, 11/12 adenocarcinoma and 1/12 adenosquamous carcinoma; and 8/12 EGFR exon 19 or 4/12 exon 21 mutation, coexisted with the EML4-ALK variant 1 (7/8) or variant 6 (1/8). A favorable response was observed according to image analysis following treatment with gefitinib for 8 months in the present case study. However, the results reported in the literature are inconsistent and the appropriate treatment for this subset of patients with NSCLC remains to be elucidated.

Finally, the present study had certain limitations. Firstly, the primary lung tumor specimen is no longer available, therefore, our results cannot be further verified in the primary tumor, with the possible mechanism of drug resistance, including primary drug resistance and acquired resistance remaining unknown. Secondly, the response of the ALK inhibitor in this patient is unknown since no ALK-targeted agents were used.

In conclusion, the present study reported a rare case of lung cancer, harboring both the *EGFR* mutation and the EML4-ALK fusion gene. Treatment with gefitinib has demonstrated a good response thus far. Future research and experience are required to understand the biological features and the optimal targeted treatment modes for this subtype of patients with lung cancer.

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