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Case Report
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EML4-ALK (E13:A20) and GPAT3-ALK (G3:A20) Mutations from a Patient with Lung Adenocarcinoma

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Abstract

Background: Anaplastic Lymphoma Kinase (ALK) gene rearrangements appear in 5% to 7% lung adenocarcinoma. With the development of Next-Generation Sequencing (NGS), increasing ALK gene variation (rearrangement/fusion, mutation or amplification) has been discovered. The result of NGS, which could predict the sensitivity of TKI drugs, shows a good correlation with tumor development and therapeutic effect.

Case Report: Herein, we report the case of a 49-year-old man who was diagnosed with lung adenocarcinoma and was undergoing ensartinib treatment. The analysis of NGS detected a new ALK fusion combination, namely EML4-ALK and GPAT3-ALK. According to the result of NGS, this patient has higher sensitivity to some TKI drugs.

Discussion: Different types of ALK gene mutations are common in lung adenocarcinoma and different degrees of influence on tumors. This case report demonstrates the effectiveness of using NGS to discover novel ALK fusion genes and guide individual therapy.

Keywords: Lung adenocarcinoma; ALK fusion; Target therapy; Ensartinib

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Introduction

Anaplastic Lymphoma Kinase (ALK) gene variations (rearrangement/fusion, mutation or amplification) have been found in a variety of tumors. Second only to EGFR mutation, ALK variation is the major explicit cause of tumor progression in non-small cell lung cell(NSCLC), accounting for about 2% to 7% in NSCLC patients. And it is most often found in young non-smoking lung adenocarcinoma patients. NSCLC harboring ALK rearrangements (fusions) are sensitive to ALK inhibitors. While NSCLC harboring certain ALK kinase region acquired mutations (e.g., L1196M, G1269A, G1202R, T1151dup, l1152R, C1156Y, F1174L, etc.) or copy number amplifications may be resistant to the early generation ALK inhibitor-crizotinib, but be sensitive to a new generation of ALK inhibitors [1]. NGS could help us to detect the new fusion genes of ALK accurately and guide us to understand the significance of ALK fusion genes in NSCLC. Previous studies have found that different fusion subtypes of ALK can affect patients' sensitivity to disparate TKI drugs. For example, the mutation of P.il1171ASN (C.3512T>A) can induce drug resistance to crizotinib and alectinib, but not to seritinib. The mutation of P. tHR790MET is the main mechanism of TKI resistance (C.2369C>T) [2].

More than 30 ALK rearrangement partners, including EML4, FAM179A, STRN, KLC1, TACR1, HIP1, CRIM1, DTSF, ITGAV, TTC27, STK17B, C12orf75, FUT8, CLTC, EPAS1, ATP13A4, LYPD1, CAMKMT, TANC1, LI NC00327, LIMD1, LOC102467213, etc. have been found, including single ALK rearrangement and multiple ALK rearrangements. More novel ALK fusion genes are also constantly being discovered [3]. For EML4-ALK fusion genes, it can be divided into “long” variants (containing TAPE domain) and “short” variants (not containing TAPE domain). Studies have proved that the "long" variant of EML4-AlK creates structural instability and has a better effect on ALK TKIs [4]. However, the effect of targeted drugs on other fusion subtypes remains unclear. Nowadays, single rearrangement seems to account for the majority of ALK fusions, and the impact of single ALK rearrangement and multiple ALK rearrangements on patient survival is still controversial.

Perhaps, whether the participation of EML4-ALK fusion genes is a significant factor for the result [3]. According to Standards and Guidelines of Sequence Variants in Cancer, single target detection methods have been replaced by Next-Generation Sequencing (NGS) or massively parallel sequencing [4]. For NGS, it can evaluate multiple genes simultaneously. In addition, high efficiency, low cost and high amount of information are also its advantages. Interpretation of NGS results will also provide a reference value for clinical treatment [4,5].

Here, we report a novel NGS discovery of the coexisting fusion of EML4-ALK (E13:A20) and GPAT3-ALK (G3:A20) from a patient with lung adenocarcinoma. In this case, the patient contains an EML4-ALK V1 (E13:A20) long mutation and a new GPAT3-ALK (G3:A20) fusion gene, which indicates that it has higher sensitivity to some TKI drugs.

Case Presentation

A 49-year-Chinese male patient who had been smoking for 3 years in the previous 20 years. Two uncertain pathologic nodules (0.6 cm in left lung upper lobe and 1.1 cm in right lung lower lobe) and multiple small solid nodules were found in the lung by Computed Tomography (CT) in 2020. The patient had no specific symptoms including fever, cough, expectoration, chest pain, fatigue and other symptoms. He reviewed the chest CT scan in March 2021, and the CT revealed that the solid nodules in the lower lobe of the right lung are slightly larger than before and the mediastinal lymph nodes are also increased slightly, while there was no obvious change in the left upper lobe nodule. In May 2021, he underwent surgical resection of right lung mass, with a diagnosis of lung adenocarcinoma and Immunohistochemistry (IHC) staining showed positive expression of TTF-1, CK7, CK, Napsina, Ki-67 (20%) and negative expression of cgA, p40, syn, CK5/6 (Figure 1). The patient also performed an NGS in Burning Stone Biotechnology (Guangzhou, China) and the sequencing results identified two fusion genes: EML4-ALK (E13:A20) fusion (abundance: 2.92%) and a new GPAT3-ALK (G3:A20) fusion (abundance: 1.01%) (Figure 2). According to sequencing results, the patient underwent ensartinib treatment after surgery (225 mg, once a day, expected for 12 months).

Discussion

In this case report, based on NGS sequencing results, we found two ALK gene rearrangements (EML4-ALK and the new GPAT3-ALK) in the patient's postoperative tumor samples. As a new detection method, NGS can find some rare variants and provide reliable evidence for treatment. For lung adenocarcinoma patients, single rearrangement and multiple rearrangements both have been previously reported [3]. A retrospective experiment concluded that among 90 patients with lung adenocarcinoma subjected to NGS, except for rearrangement-free patients, there are 73 patients with ALK single rearrangement and 16 patients with multiple rearrangements. However, there is no obvious difference in survival between the two rearrangement types. But another retrospective study concluded that patients with a single EML4-ALK fusion had a better PFS than those with multiple fusions [6]. Since ALK fusion is not grouped, the controversial conclusion may be due to whether there is involvement of the EML4-ALK fusion gene [3]. This patient is still in the early stage of treatment, so the prognosis is not clear. In addition to lung adenocarcinoma, we can also detect the presence of EML4-ALK fusion genes in other tumors, such as breast cancer and colon cancer. The fusion of EML4 and ALK kinase domain leads to abnormal signal transduction, which increases cell growth, proliferation and cell survival [7]. According to different variants number, the EML4-ALK rearrangements can be divided into EML4-ALK V1 (E13:A20), EML4-ALK V2 (E20:A20), EML4-ALK V3A/B (E6:A20), EML4-ALK V4 '(E15:A20) and EML4-ALK V5 A/B (E2:A20), EML4-ALK V5 '(E18:A20), EML4-ALK V7 (E14:A20), EML4-ALK V8 A/B (E17:A20), etc [8]. All EML4-ALK variants contain the entire intracellular kinase domain of ALK, encoded by exons 20 to 29. Although they all express the N-terminal helical trimerization domain, they do not always express the same length of the tandem atypical β-propeller domain (TAPE), which leads to differences in different EML4 genes [3,6]. According to

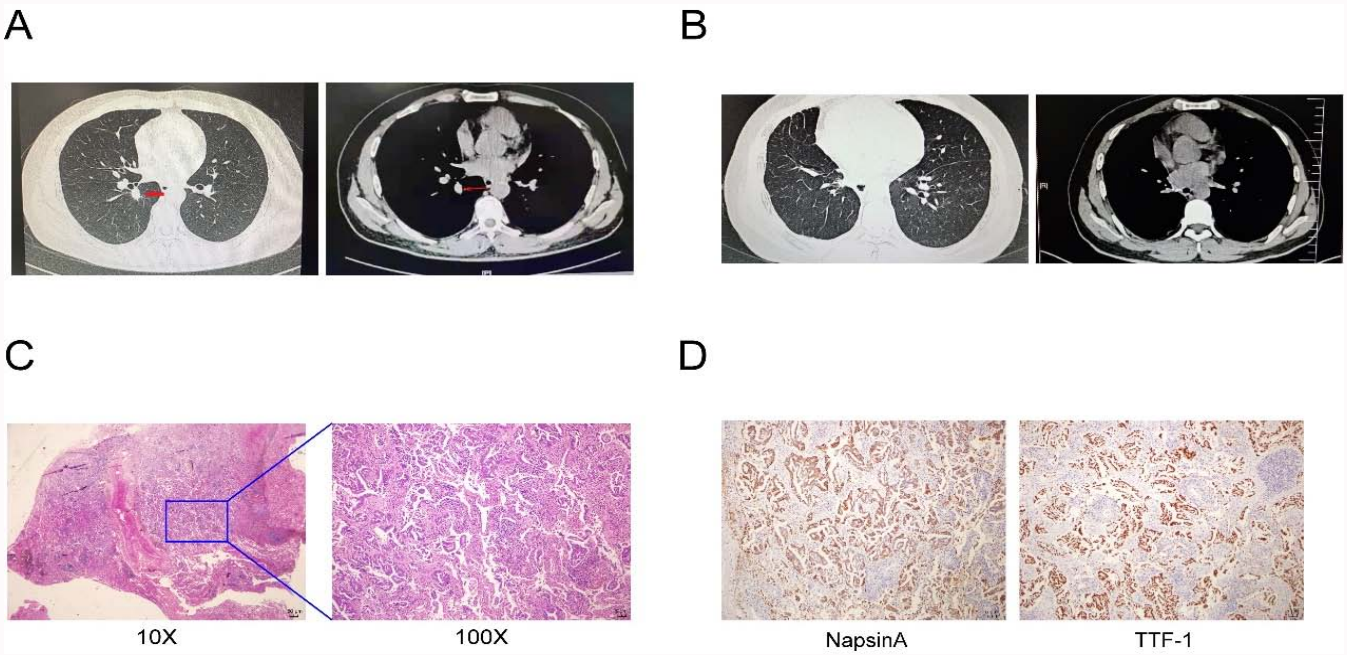

Figure 1: CT scans and Histological findings of the patient. (A) CT scans of the patient before operation. (B) CT scans of the patient after operation. (C) HE staining showed lung adenocarcinoma (L: 10x; R: 100x). (D) IHC: NapsinA and TTF-1 (100x).

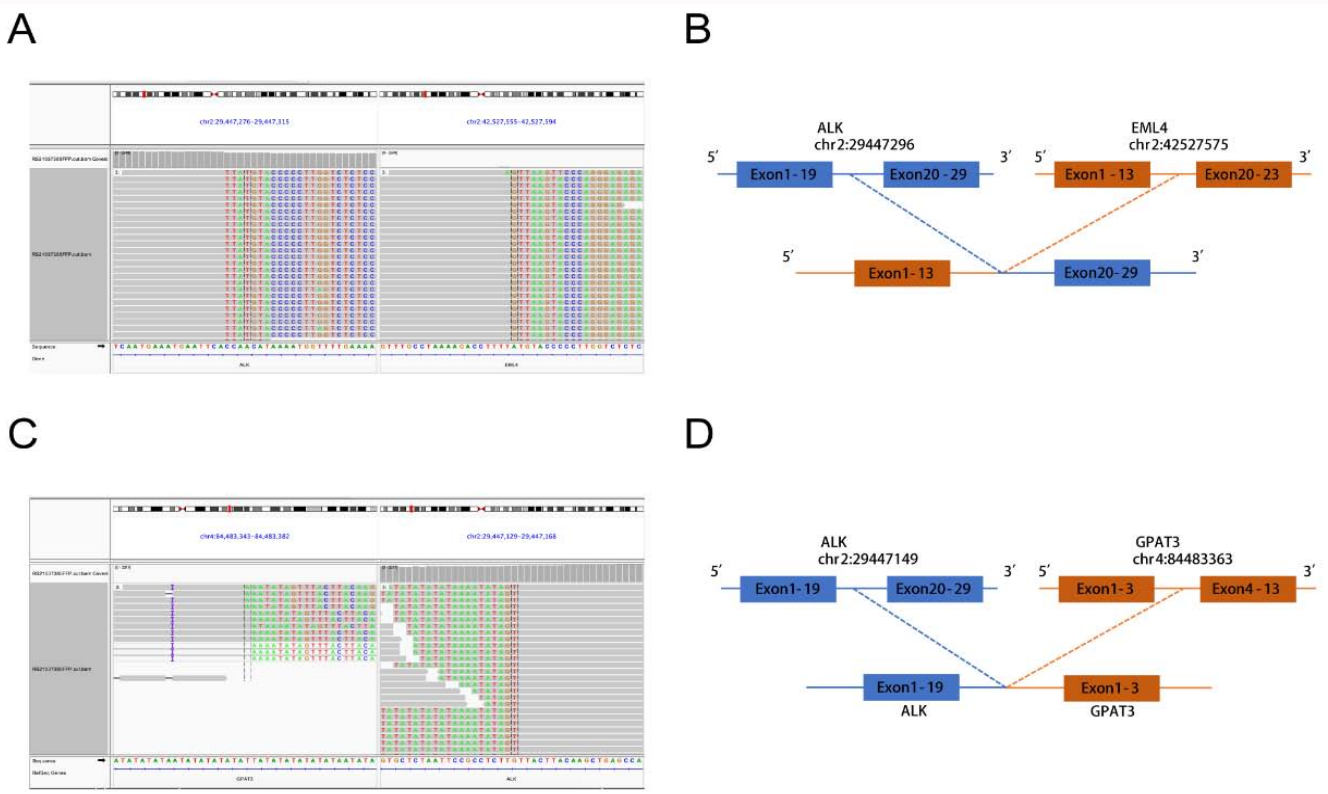

Figure 2: Next-generation sequencing findings from tumor sample. (A) EML4-ALK fusion in the present case visualized using the Integrative Genomics Viewer (IGV). (B) Diagram depicting the EML4-ALK fusion. (C) GPAT3-ALK fusion in the present case visualized using the Integrative Genomics Viewer (IGV). (D) Diagram depicting the GPAT3-ALK fusion.

Table 1: Genetic information for EML4 and GPAT3, from NCBI.

Official Symbol	Full Name	Introduction
EML4	EMAP like 4	This gene is a member of the echinoderm microtubule associated protein-like family. The encoded WD-repeat protein may be involved in microtubule formation. Abnormal fusion of parts of this gene with portions of the anaplastic lymphoma receptor tyrosine kinase gene, which generates EML4-ALK fusion transcripts, is one of the primary mutations associated with non-small cell lung cancer. Alternative splicing of this gene results in two transcript variants.
GPAT3	glycerol-3-phosphate acyltransferase 3	This gene encodes a member of the lysophosphatidic acid acyltransferase protein family. The encoded protein is an enzyme which catalyzes the conversion of glycerol-3-phosphate to lysophosphatidic acid in the synthesis of triacylglycerol. Multiple alternatively spliced variants, encoding the same protein, have been identified.

the existence of TAPE domain or the length of TAPE, EML4-ALK variants can be divided into "long" (V1, V2, V4 ', V5 ', V7, V8 A/B, etc.) and "short"(V3 A/B, V5 A/B) variants, which play different roles in clinical function [6]. In this case, EML4-ALK (E13:A20) was a "long" variant that may predict a better response to the small molecule Tyrosine Kinase Inhibitor (TKI). Based on the relevant data statistics, EML4-ALK V3 A/B (E6; A20) accounted for 42%, followed by 37% of EML4-ALK V1 (E13:A20) [8]. With the continuous application of second-generation sequencing, more EML4-ALK variants with a frequency of less than 1% have been discovered. In addition, the number of gene rearrangements and partner types can also affect the efficacy of some TKI drugs [9]. For example, there has been a report that crizotinib might have a better effect on the OFCC1-ALK fusion gene [10]. And the fusion variants with the TAPE domain are also sensitive to HSP90 inhibitors. In this patient, we also found GPAT3-ALK (G3:A20) fusion as the second variant gene, and the detailed information about the two genes are shown in Table 1. GPAT3 is mainly expressed in adipose tissue, followed by testis and kidneys [11]. Studies have found that GPAT3 is highly expressed in endometrial cancer high-risk groups [12]. Existing studies suggest that GPAT1 and GPAT2 are located in mitochondria, and GPAT3 and GPAT4 are located in the endoplasmic reticulum. Some studies have shown that GPAT2 is highly expressed in melanoma, lung cancer, prostate cancer and breast cancer, promoting cell proliferation, anchorage-independent growth, migration and survival of tumor cells [13,14]. It can also participate in piRNA metabolism and regulate the cell cycle [15]. Targeted therapy for GPAT3 has not been reported. With the development of NGS technology, the NGS results obtained from DNA or RNA extracted from tumor tissue can show its complex molecular characteristics. In this case report, sequencing results showed that EML4-ALK and GPAT3-ALK fusion genes could affect the sensitivity of targeted drugs, as detailed in Table 2. According to the sequencing results, we can choose drugs with high sensitivity for treatment. For this patient, we chose ensartinib for treatment. According to a recent Systematic Review and Network Meta-Analysis, ensartinib has the better PFS than other TKIs [1]. The effect of alectinib on PFS is only inferior to ensartinib, but it has the best ORR in Asian ALK-rearranged NSCLC patients. Horn

Table 2: Interpretation of mutations from NGS.

Variation results	Targeted drugs (Sensitivity, level of evidence)
EML4-ALK(E13:A20)	Aletinib (Sensitive, Grade A); Crizotinib (Sensitive, Grade A); Ceritinib (Sensitive, Grade A); Brugatinib (Sensitive, Grade A); Lauratinib (Sensitive, Grade A); Ensatinib (Sensitive, Grade A); Entritinib (Sensitive, Grade C)
GPAT3-ALK(G3:A20)	Aletinib (Sensitive, Grade A); Crizotinib (Sensitive, Grade A); Ceritinib (Sensitive, Grade A); Brugatinib (Sensitive, Grade A); Lauratinib (Sensitive, Grade A); Ensatinib (Sensitive, Grade A); Entritinib (Sensitive, Grade C)

reported an analysis of the eXalt3, a global phase 3 study of ensartinib *vs.* crizotinib in patients with ALK-positive NSCLC who had not previously received ALK inhibitor treatment [16]. Ensartinib has the better effect on systemic and intracranial diseases and the higher ORR and PFS [17]. Meanwhile, ensartinib has better tolerance [18]. Considering that patient is in the early stage of treatment, the efficacy of targeted therapy needs to be further analyzed and confirmed. With the continuous progress of sequencing technology, more rare fusion and gene variations will be found. Newly discovered fusion genes like GPAT3-ALK, with the continuous expansion of the sample size that has undergone NGS, the impact of different fusion genes of ALK on the pathogenesis and the effect of treatment will be further clarified. For various ALK kinase region acquired mutations and combination types, different ALK inhibitors have different sensitivities for different sites. Therefore, retesting after ALK inhibitor resistance is important for the precision and individualized treatment of patients with ALK fusion NSCLC.

In summary, we discovered a new ALK coexistence fusion combination through powerful NGS: EML4-ALK (E13:A20) and GPAT3-ALK (G3:A20), which expands the ALK fusion range in NSCLC. In addition, analysis of the NGS results can also provide a reliable basis for future treatment of NSCLC.

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