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A novel intergenic region *ALK* fusion is targetable by alectinib in a non-small cell lung cancer patient with brain metastasis

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Anaplastic lymphoma kinase (*ALK*) rearrangement defines a unique nonsmall cell lung cancer (NSCLC) molecular subtype, of which the patients could potentially benefit from anti-*ALK* therapies. So far, the outcomes of the canonical echinoderm microtubule-associated protein-like (*EML-ALK*) patients subjected to *ALK* inhibitors are well established. However, given the increasing complexity of *ALK* fusion partners, as detected by high-throughput sequencing, the responses of those with rare *ALK* fusion events remain to be explored. Here, we report a lung adenocarcinoma patient with brain metastasis harboring an *ARHGAP5* downstream intergenic region *ALK* fusion, as detected by using DNA-based next-generation sequencing, who experienced a partial response to alectinib treatment. While whole- transcriptome RNA sequencing (RNA-seq) failed to identify potential ALK fusion transcripts, subsequent targeted deep RNA-seq revealed the expression of *EML4-ALK* transcripts in the tumor tissue. Given the increasing application of the ALK-tyrosine kinase inhibitors (TKIs), it is extremely crucial to define the patients who could be suitable for this treatment in clinic. The present case has provided supporting evidence that noncanonical *ALK* rearrangements on the genomic level are often functionally relevant and targetable by ALK-TKI, particularly in cases with sub-optimal quantity and quality for RNA validation.

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Introduction

Anaplastic lymphoma kinase (*ALK*) rearrangement occurs in about 3–7% patients with non-small-cell lung cancer (NSCLC) [1,2]. Echinoderm microtubule-associated protein-like (*EML4*) represents the most common fusion partner of *ALK*, accounting for about 90% of the *ALK*-positive NSCLC cases [3]. The advances in next-generation sequencing (NGS) technologies have enabled the identification of increasingly more rare and novel *ALK* fusion partner genes, including *CUX1-ALK*, *GCC2-ALK* and *TNIP2-ALK* [4–6], against which *ALK* tyrosine kinase inhibitors (TKIs) have demonstrated activity and favorable clinical outcome. To date, the clinical effect of crizotinib, the first-generation *ALK* TKI, against most uncommon forms of *ALK* fusions has been well established. In comparison with crizotinib, alectinib exhibited higher efficacy and tolerability, as well as superior central nervous system (CNS) activity [7]. In this case, we report a lung adenocarcinoma patient with brain metastasis, who carried an intergenic *ALK* rearrangement as detected by DNA-based NGS, and showed a favorable clinical response to alectinib.

Case presentation

A 55-year-old Chinese male with no smoking history came into our hospital with a severe headache in June 2020. MRI scanning revealed a space-occupying lesion in the left parietal lobe of the brain (Fig. 1a). Presurgical physical examination detected nodules in the right upper lung lobe by using computed tomography (CT) (Fig. 2a). The tumor lesions in the brain and lung were removed by surgery in June and July 2020, respectively. No residual tumors were detected postsurgery by Cranial MRI (Fig. 1b) and chest CT (Fig. 2b). Pathological analysis of the surgical brain and lung tissue samples showed poorly differentiated lung adenocarcinoma with a clinical stage of T4N2M1 (IVB). The surgical margin was tumor cell-negative, and the blood tumor markers, including carcinoembryonic antigen (CEA), neuron-specific

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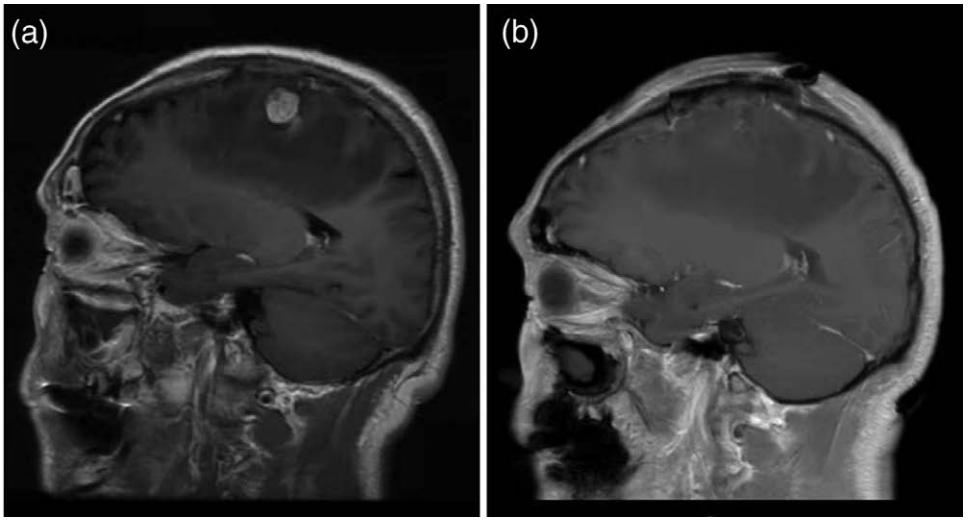
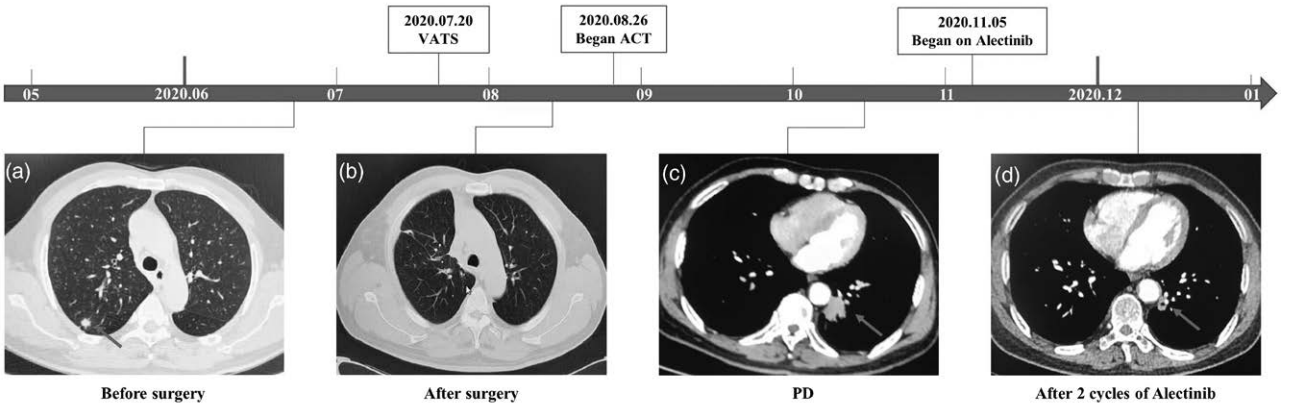
Fig. 1



Representative radiologic images before and after surgery. Brain metastases in the left parietal lobe before surgery (a) and after surgery (b).

Fig. 2

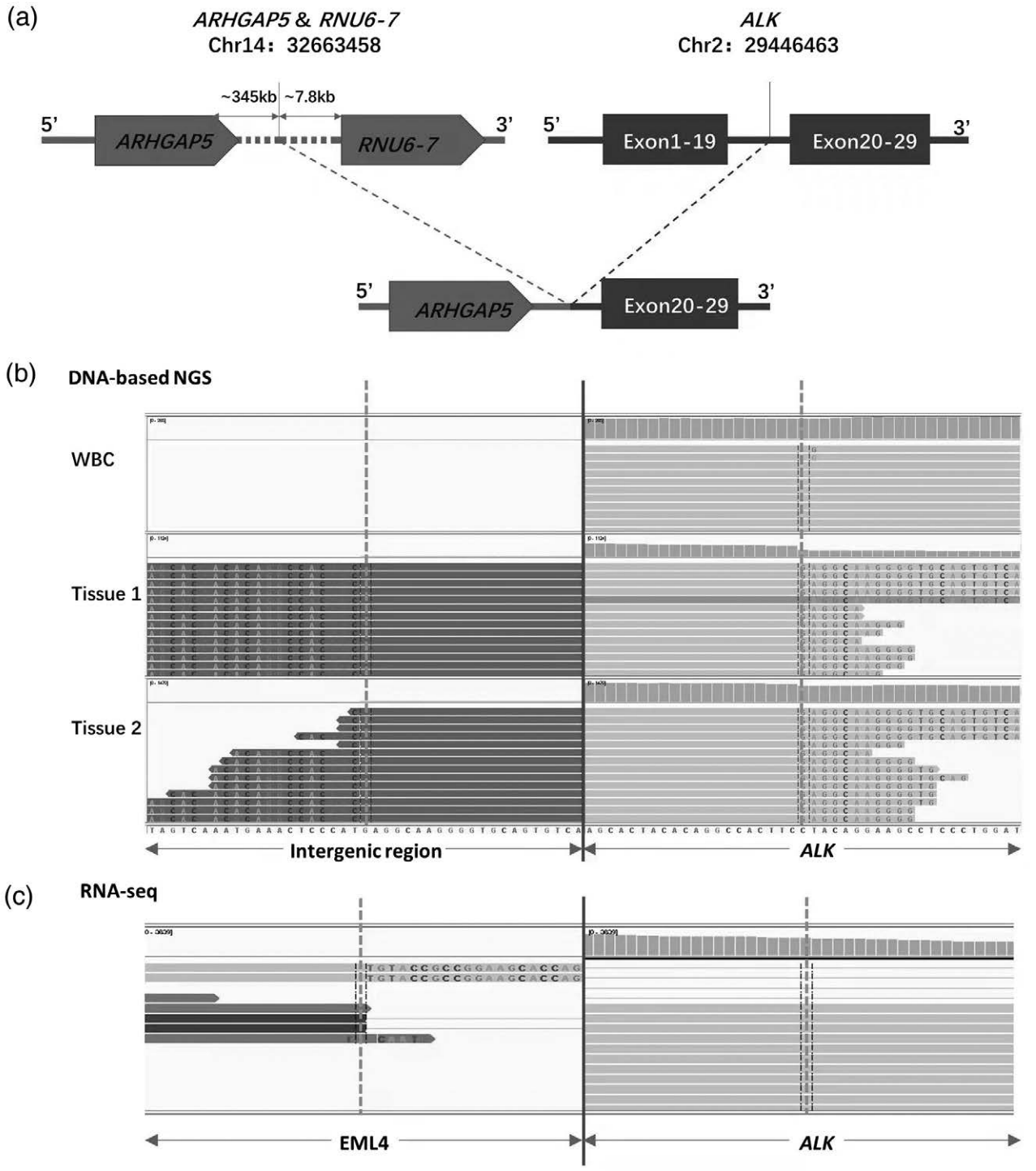


Representative radiologic images and treatment timeline of the patient. The red arrows indicate the primary tumor lesions in the lung before surgery (a), after surgery (b), at disease progression (c) and regression after alectinib treatment (d).

enolase and CA199, were all in the regular ranges following surgical resection. However, after two cycles of adjuvant therapy (pemetrexed plus carboplatin), CT scanning found an emerging nodule in the left lower lobe of the lung (Fig. 1c), and the blood CEA increased to 20.8 ng/mL.

To search for potential treatment options, DNA-based NGS testing was performed on the formaldehyde-fixed paraffin-embedded (FFPE) surgical samples of the brain and lung. We identified an intergenic *ALK* rearrangement, with the intergenic region (IGR) downstream of *ARHGAP5* fused to exon 20 of *ALK* (Fig. 3a,b). This is a novel form of fusion that retains the full ALK kinase domain. No additional driver alterations were detected. Considering the patient had brain metastasis and alectinib has superior systemic and CNS activity, the patient was subjected to oral alectinib therapy at the dose of 600 mg twice daily. After two cycles of treatment, CT scans observed a significant shrinkage of the lesion in the lung (Fig. 1d) and the CEA level decreased to 1.38 ng/mL. To identify the expressed fusion gene, we first performed whole transcriptome RNA-seq in both the primary tumor and brain metastasis lesion, yet failed to identify *ALK* fusion transcripts. To better enrich for cancer gene transcripts, targeted RNA-seq was performed by using the remaining primary tumor sample and revealed typical *EML4-ALK* (ex13:ex20) fusion transcripts (Fig. 3c). The patient continued to experience partial response and remained progression-free on oral alectinib therapy for over 17 months up to present.

Fig. 3



A novel intergenic region between *ARHGAP5* and *RNU6-7* fused to the *ALK* kinase domain was identified. (a) The schematic of the novel *ALK* fusion. The breakpoints on the intergenic region and *ALK* are located on chr14:32663458 and chr2:29446463, respectively. The snapshots of the *ALK* fusion tested by DNA-based NGS (b) and RNA-seq (c) on Intergrative Genomics Viewer. The dashed lines in green indicated the breakpoints. The colored letters indicate the mismatched bases around the breakpoints due to the *ALK* rearrangement. ALK, anaplastic lymphoma kinase; NGS, next-generation sequencing.

Discussion

To our best knowledge, this is the first case of a lung adenocarcinoma patient harboring an intergenic *ALK* rearrangement as detected by DNA-based NGS testing and was successfully treated with alectinib. The wide use of NGS testing has facilitated the detection of uncommon *ALK* fusions, including those with one IGR breakpoint [8,9]. A previous study has demonstrated that among the conventional *ALK* testing methods, DNA-based NGS and fluorescence in situ hybridization have the uppermost positive rates (92.7% and 94.5%, respectively) than IHC [10] (87.3%). While IGR-*ALK* fusions at the genomic level may not necessarily result in detectable fusion transcripts [11], many studies have shown that the noncanonical *ALK* fusions at the DNA level could generate functional fusion as a result of mRNA splicing. For instance, it has been reported that the *SLC19A3* IGR-*ALK* and the *ASXL2-ALK* fusions result in the variant 1 *EML4-ALK* (ex13:ex20) transcripts at the RNA level [12,13]. The *C2orf91-ALK* fusions can form variant 2 transcripts *EML4-ALK* (ex20:ex20) [14]. Moreover, the *CCDC85A-ALK* can generate variant 3 *EML4-ALK* (ex6:ex20) transcripts [14]. As emerging data have demonstrated the variable clinical response of *ALK* fusion subtype/variants to targeted therapies [15], our case further supports the use of DNA testing combined with RNA-seq verification. However, due to the limited quality and quantity of RNA in clinical FFPE samples, our case suggests that targeted deep RNA-seq may be necessary to identify patients who could benefit from ALK TKI treatment.

In summary, DNA-based sequencing enables rapid and reliable detections of driver events that are caused by various mutation types in cancer patients. Our case suggests that IGR-*ALK* fusions at the DNA level might be equally informative with respect to the choice of *ALK* TKIs and a combination of DNA and targeted RNA sequencing tests could further pinpoint the exact *ALK* fusion genes in NSCLC patients for targeted therapies.

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The data in the current study are not publicly available to protect patient privacy, but the data are available upon reasonable request.

The patient declared to consent to participate in this study. The patient declared to consent to the publication of the current article.

Conflicts of interest

There are no conflicts of interest.

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