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Identification of a novel *WNK1–ROS1* fusion in a lung adenocarcinoma sensitive to crizotinib

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ABSTRACT

Objectives: Non-small-cell lung cancer (NSCLC) has various driver mechanisms, including *ROS1* rearrangement with different fusion patterns. There is a need to identify and evaluate new *ROS1* fusions and the response to targeted therapy.
Materials and methods: A targeted next-generation sequencing (NGS) panel was used to analyze DNA extracted from tumor tissue and blood samples from an NSCLC patient. Results were validated using Sanger sequencing.
Results: We found a novel *ROS1* rearrangement form, namely a *WNK1–ROS1* fusion. The transmembrane and kinase domains of ROS1 remained intact in this fusion. No *EGFR*, *MET*, *KRAS*, *ALK*, *ROS1* or other NSCLC driver mutations were detected in the patient. The patient achieved a partial response after treatment with crizotinib. When disease progressed, *ROS1 G2032R* mutation—a classical mechanism of crizotinib resistance—was detected in the DNA sample extracted from the patient's plasma sample.
Conclusion: We identified a novel *WNK1–ROS1* fusion that was sensitive to crizotinib and developed an *ROS1 G2032R* mutation when the disease progressed. The *WNK1–ROS1* rearrangement appeared to be a novel driver of the lung cancer.

1. Introduction

ROS1 is a tyrosine kinase receptor that has undergone genomic rearrangements in a subset of patients with non-small-cell lung cancer (NSCLC) as well as ovarian, gastric, colorectal, and other cancers [1]. Approximately 2% of adenocarcinomas of the lung have *ROS1* fusions [2]. Fusion partners for *ROS1* include *CD74*, *SDC4*, *EZR*, *SLC34A2*, *TPM3*, and *CCDC6* [3,4]. More *ROS1* fusion partners remain to be identified and assessed.

Functional fusion of two genes creates a protein that can be a strong driver for oncogenesis. The cancer driver from a fusion often results from constitutive kinase activation. There are several mechanisms of such activation that include dimerization/oligomerization of the kinase, increased kinase expression, conformational changes favoring the active state, and loss of autoinhibitory domains [1].

It has been demonstrated that targeting therapy using small molecules directed at the constitutively activated kinase is remarkably effective. Crizotinib has multiple kinase inhibitory activities against ALK, MET, and ROS1 [5], and was approved by the Food and Drug Administration (FDA) in 2016 to treat metastatic NSCLC patients with *ROS1* fusions. Like other tyrosine kinase inhibitors, acquired resistance to crizotinib develops after treatment, including at the solvent front G2032R mutant of ROS1. The G2032 alpha carbon engages a van der Waals interaction with the pyrazole ring of crizotinib. Conformational change introduced by the G2032R mutation may interfere with the binding of crizotinib and hence confer its inhibitory activity [6].

2. Case description

A 39-year-old Chinese female was diagnosed with lung adenocarcinoma with lymph-node and brain metastases. Molecular tests showed wild-type *EGFR* exon 18–21 and *KRAS* exon 2, and ALK-negative staining. She underwent chemotherapy with bevacizumab, pemetrexed, and carboplatin for six cycles followed by eight cycles of maintenance therapy with bevacizumab and pemetrexed. Disease was progressed at the end of the maintenance chemotherapy.

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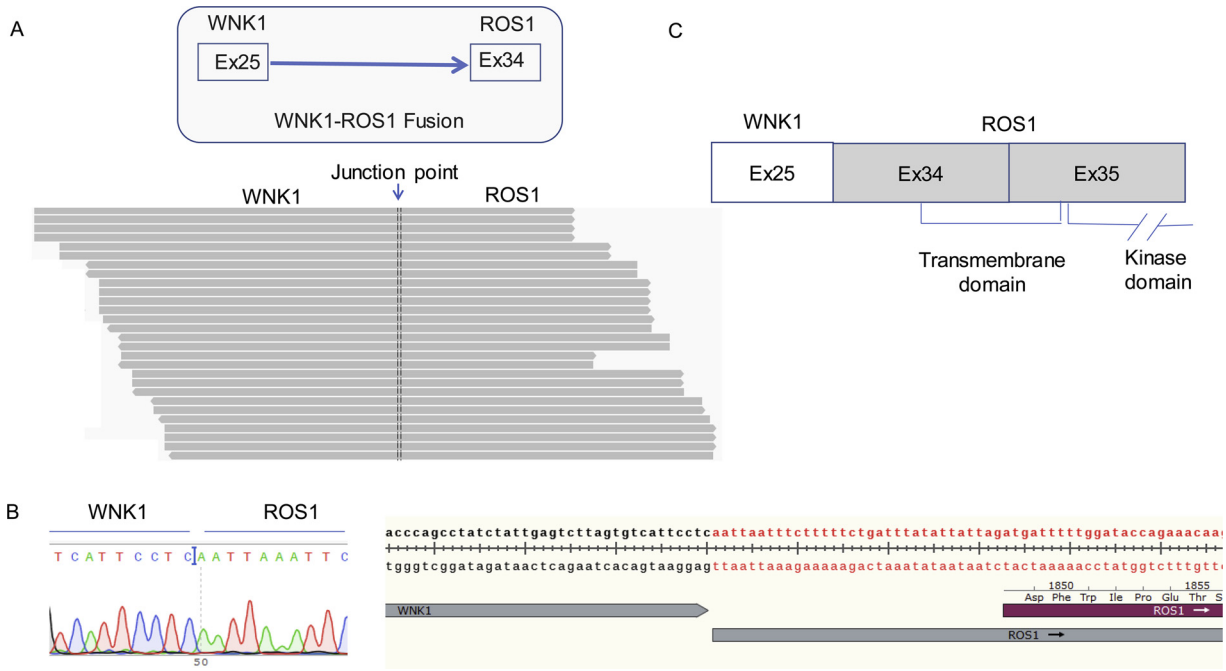

Fig. 1. Identification and verification of a novel *WNK1–ROS1* fusion. (A) Next-generation sequencing (NGS) analysis identified *WNK1–ROS1* fusion in the cancer tissue of the patient with lung adenocarcinoma. (B) Sanger confirmation of the novel *WNK1–ROS1* fusion. (C) Intact ROS1 transmembrane and kinase domains after the fusion.

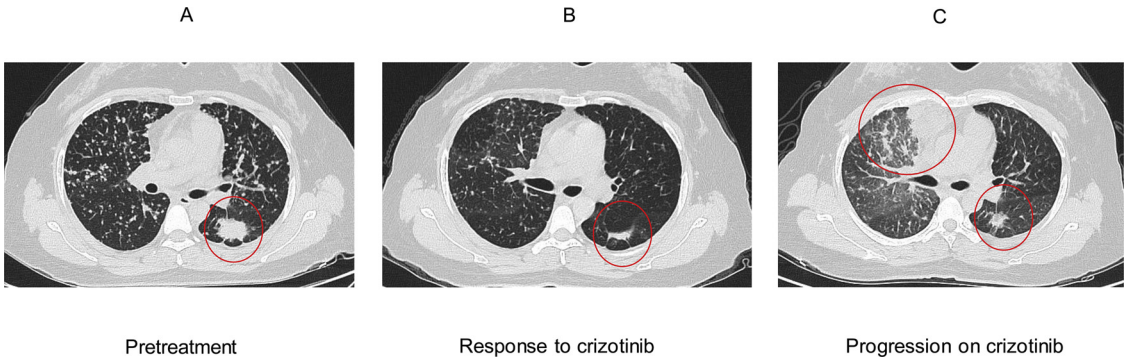

Fig. 2. Response of the patient with non-small-cell lung cancer (NSCLC) to treatment with crizotinib. (A) Prior to treatment with crizotinib. (B) Partial response achieved 3 months after crizotinib treatment. (C) Progression 5 months after crizotinib treatment.

During the course of chemotherapy, a report of positive detection of ROS1 by immunohistochemical analysis was received. After chemotherapy, a formalin-fixed, paraffin-embedded (FFPE) sample from the patient's lung cancer tissue was extracted and analyzed using a targeted NGS panel. A novel rearrangement of *ROS1*—namely a *WNK1–ROS1* fusion—was identified (Fig. 1A). The sample exhibited a 19.3% *WNK1–ROS1* fusion frequency. Sanger sequencing of the DNA extracted from the FFPE sample confirmed the *WNK1–ROS1* fusion (Fig. 1B). The fusion did not appear to affect the transmembrane or kinase domains of ROS1 (Fig. 1C). The NGS panel analysis showed no *EGFR*, *MET*, *KRAS*, *ALK*, *ROS1* or other NSCLC driver mutation in this patient. After identification of the *ROS1* fusion, the patient was treated with crizotinib for 3 months and achieved a partial response (compare Fig. 2A and B).

Five months after crizotinib treatment, the disease progressed again (Fig. 2C). A blood sample from the patient was extracted and analyzed by NGS. *ROS1 G2032R* mutation, at 4.6% frequency, was identified from the circulating tumor DNA (ctDNA) obtained after the patient had developed resistance to the ROS1 tyrosine kinase inhibitor (Fig. 3); this is consistent with the mechanism of resistance to TKI treatment [1].

3. Discussion

We found an NSCLC patient harboring a novel *WNK1–ROS1* rearrangement that was sensitive to crizotinib treatment; *ROS1 G2032R* mutation developed when the disease progressed.

ROS1 rearrangement generally occurs in the absence of other known oncogenic drivers [8]. Consistently, we did not observe driver mutations in *EGFR*, *KRAS*, *MET* or *ALK* fusion in this case study. The patient's initial response to crizotinib and subsequent development of *ROS1 G2032R* mutation suggests that the adenocarcinoma the patient developed was a result of the activation of the ROS1 pathway. The patient is younger and a non-smoker, which also fits the clinicopathological characteristics of *ROS1* fusion [7].

Fusion that leads to constitutive kinase activation can be a strong driver for oncogenesis [1]. Several *ROS1* fusion partners have been identified, including *CD74*, *SDC4*, *EZR*, *SLC34A2*, *TPM3*, *LIMA1*,*MSN*, *GOPC*, *CCDC6*, and *CEP85L* [3,4,7]. Many *ROS1* fusion partners have no dimerization domains, and the mechanism of constitutive ROS1 protein action is unknown. ROS1 fusion generally results in autophosphorylation of ROS1 and the downstream signaling of MEK, ERK, STAT3, and AKT, which are blocked by ROS1 inhibition [7]. The

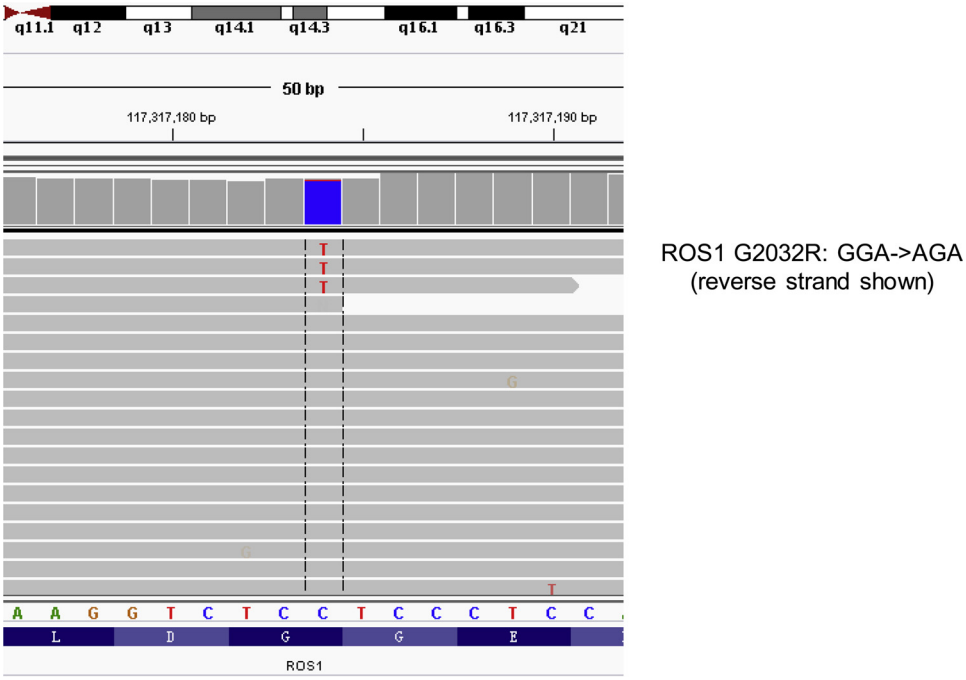

Fig. 3. Development of *ROS1 G2032R* mutation. Integrative genomics viewer (IGV) view of the next-generation sequencing (NGS) results in circulating tumor DNA (ctDNA) of the patient after developing resistance to crizotinib.

downstream oncogenic pathway may differ depending on the fusion partner. As an example, CD74–ROS1 fusion activates a novel cancer invasion pathway through E-Syt1 phosphorylation [8]. A patient with a *ROS1* fusion had a median response duration of 17.6 months with crizotinib treatment. However, some patients had a durable response of only a few months [3]. The relationship between *ROS1* with different fusion partners and the response to crizotinib remains to be assessed.

WNK1 belongs to a family of serine/threonine kinases that play key roles in ion homeostasis in the kidney and nervous system [9]. A chimeric transcript WNK1– B4GALNT3 has been identified in papillary thyroid carcinoma and correlated with B4GALNT3 overexpression [10]. In line with this observation, tumor tissue with the *WNK1–ROS1* fusion identified in the case study exhibited positive staining of ROS1 by IHC; this is consistent with increased ROS1 kinase expression as a potential mechanism for the oncogenic effects driven by the *WNK1–ROS1* fusion.

Like other small-molecule inhibitors, resistance to drugs targeting fusion occurs. The mechanism of such resistance includes 'on-target' mutation/amplification of the fusion itself, and 'off-target' activation of parallel bypass pathways [1]. For ROS1-positive NSCLC, the most common mechanism of resistance to crizotinib is ROS1 G2032R mutation that leads to steric interference with drug binding analogous to the ALK G1202R mutation. Consistent with this, we observed *ROS1 G2032R* mutation after the patient developed resistance to crizotinib (Fig. 3).

In this study we identified a patient with a novel *ROS1* fusion who was sensitive to crizotinib treatment. We also detected crizotinib-resistant mutant *ROS1 G2032R* in the patient's plasma sample, supporting the concept of real-time monitoring of the drug effect using liquid biopsy. Further work is required to validate the utility of circulating tumor DNA as a tool to monitor treatment efficacy and resistance to drugs targeting chromosome rearrangement.

Conflict of interest statement

NL, TW, YP, and RL are employees of Hangzhou Repugene Technology Co., Ltd.

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