Case Report
Effectiveness of crizotinib in a patient with novel SDC4-ROS1 fusion variant (E5:E34) pleural metastatic lung adenocarcinoma

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Abstract: The c-ros oncogene 1 (ROS1)-rearranged non-small cell lung cancer (NSCLC) is a unique molecularly defined yet heterogeneous subset of NSCLC. We report a case of crizotinib effectiveness in a pretreated patient with a metastatic NSCLC for ROS1 rearrangement. Variant of SDC4-ROS1 rearrangement was identified (E5:E34) by Next-generation sequencing (NGS), which different from previously identified SDC4-ROS1 fusion variant (E2:E32, E4:E32, E4:E34). The novel SDC4-ROS1 fusion variant was involved the translocation of exons 1-5 of SDC4 to exons 34-43 of ROS1. To the best of to our knowledge, this is the first case report of a patient with novel SDC4-ROS1 fusion variant (E5:E34) fusion. This patient had an excellent response to crizotinib, suggesting that the SDC4-ROS1 fusion oncogenic driver.

Keywords: Lung cancer, SDC4-ROS1 fusion variant, crizotinib, next-generation sequencing

Introduction
The c-ros oncogene 1 (ROS1) fusions occur in approximately 1-2% of non-small cell lung cancers (NSCLC) [1, 2], it has been demonstrated that crizotinib, a multi-targeted ALK/ROS1/ MET tyrosine kinase inhibitor (TKI), has significant clinical activity in ROS1-rearranged non-small-cell lung cancer (NSCLC) [3]. To date, 14 fusion partners of ROS1 rearrangements have been reported in NSCLC, including CD74, SLC-34A2, GOPC, CCDC6, SDC4, TPM3, EZR, LRIG3, KDEL R2, LIMA1, MSN, CLTC, TMEM106B, and TPD52L1, with CD74 being the most common fusion partner [4]. Here we report a variant of SDC4-ROS1 rearrangement (E5:E34) in a patient with pleural metastatic lung adenocarcinoma, which different from previously identified SDC4-ROS1 fusion variant (E2:E32, E4:E32, E4:E34).

Case report
A 25-year-old Chinese young man was admitted with a 3-month history of cough and mild shortness of breath in July 2015. X-ray of the chest revealed left large pleural effusion. The patient had no smoking history. He had no family history of malignant tumor. A pleural aspiration showed pale red pleural fluid. This fluid was determined to be an exudate with very high concentrations of CEA (355.2 ng/ML). 18F-FDG-PET/CT scan showed the left pleura significant thickening with increased FDG metabolism lesions (SUVmax of 9.0) (Figure 1A, 1B). PET/CT-positive left lung hilar and mediastinal lymph nodes were present with SUVmax of 9.3, furthermore, multiple small pulmonary nodules were found at right lower lobe and two upper lobes of sub-pleural, however, with no increased FDG metabolism. The thoracoscopy showed nodularity of the visceral and parietal pleura with significantly diffuse hyperemia. Thoracoscopic pleural biopsies showed malignant cells with morphologic of adenocarcinoma and immunohistochemically features consistent with metastatic adenocarcinoma of pulmonary origin (TTF-1 positive, AE1/AE3 positive, CEA positive, Calretinin negative, Vimentin negative, WT1 negative, CK5/6 negative) (Figure 2).
Novel SDC4-ROS1 fusion variant (E5:E34) in a patient of pleural metastatic lung

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**Figure 1.** A-H. Radiographic evidence of clinical response to crizotinib. After having undergone two systemic treatments, in November 2015 patient started crizotinib treatment, which led to deep and prolonged response upon diffuse pleural metastatic sites.

**Figure 2.** Pathological examination of thoracoscopic pleural biopsies revealed of metastatic lung adenocarcinoma by HE stain (A) and immunostaining of TTF1 (B), AE1/AE3 (C) and CEA (D) in pleural tissues.

The biopsied specimen from this patient was tested for epidermal growth factor receptor (EGFR) using amplification-refractory mutation system (ARMS) assay with AmoyDx EGFR29 Mutations Detection kit; The anaplastic lymphoma kinase (ALK) status was also tested by reverse-tran-
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Figure 3. Next-generation sequencing (NGS) data indicated a somatic intrachromosomal SDC4-ROS1 (E5:E34) fusion as demonstrated by Integrative Genomics Viewer program.
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scriptase polymerase chain reaction (RT-PCR) assay with AmoyDx EML4-ALK Fusion Gene Detection Kit (Amoy Diagnostics, Xiamen, China). The result showed that the patient was negative for EGFR mutations and EML4-ALK fusion. Therefore, the diagnosis of lung adenocarcinoma with pleural and lymph node metastasis and wild-type for EGFR and negative for rearrangement of ALK could be established. The treatment of the patient was initiated with pemetrexed plus cisplatin in the first-line treatment setting. After draining effusion as much as possible, patient received intrapleural perfusion of 60 mg cisplatin. Reduction of effusion was determined by type-B ultrasonography. After two cycles of this regimen, CT scans still revealed that left large pleural effusion and the hilar and mediastinal lymph nodes (Figure 1C, 1D). In order to further identify potential therapeutic targets, Next-generation sequencing (NGS) with a gene panel covering 416 cancer-related genes was performed on biopsy tissue. Libraries were prepared with Hyper Prep Kit (Kapa) and then sequenced on Hiseq 4000 NGS platforms (Illumina). Variants of SDC4-ROS1 rearrangement (E5:E34) were identified by NGS, which different from previously identified SDC4-ROS1 fusion variants (E2:E32, E4:E32, E4:E34). The novel SDC4-ROS1 fusion variant was involved the translocation of exons 1-5 of SDC4 to exons 34-43 of ROS1 (Figure 3). This fusion retains the ROS1 kinase domain which spans from amino acids 1945-2222, occurs at a recurrent ROS1 fusion breakpoint. Crizotinib, a tyrosine kinase inhibitor targeting ROS1 as well as ALK and MET, has been approved for the treatment of advanced metastatic ROS1-rearranged non-small cell lung cancer (NSCLC), so we decided to initiate crizotinib in November 2015 in this previously treated NSCLC patient. Four weeks later, the shortness of breath and cough significantly alleviated, while no adverse event occurred. Chest CT confirmed partial response with regression of pleural effusion after two months (Figure 1E, 1F). At last follow-up in September 2016, while still on crizotinib, he had good performance status and the pleural effusion disappeared (Figure 1G, 1H).

Discussion

Genetic rearrangements that fuse the kinase-domain containing regions of tyrosine kinases to the unrelated genes were found in multiple tumor types [1]. At present, 14 genes were found to fuse with ROS1 in NSCLC, including CD74, SLC34A2, GOPC, CCDC6, SDC4, TPM3, EZR, LRIG3, KDEL R2, LIMA1, MSN, CLTC, TPD52L1 and TMEM106B [2, 4]. All the fusion proteins retain the ROS1 kinase domain, but rarely its transmembrane domain [2]. The resulting ROS1 fusion kinases are constitutively activated and drive cellular transformation. ROS1 rearrangement defines a new molecular subset of NSCLC. The first large sample study conducted by Bergethon et al. demonstrated a 1.7% (18 of 1073) frequency of ROS1 in the general population with NSCLC, predominantly in patients with adenocarcinomas, of younger age, or never-smokers [5].

Here we report a novel SDC4-ROS1 fusion variant (E5:E34) pleural metastatic lung adenocarcinoma, which different from previously identified SDC4-ROS1 fusion variant (E2:E32, E4:E32, E4:E34) [6]. The SDC4 gene is composed of five exons. The protein is a transmembrane (type I) heparin sulfate proteoglycan that is found as a homodimer. It mediates numerous cellular processes through signaling pathways that affect cellular proliferation, migration, mechanotransduction and endocytosis [7, 8]. Notably, it functions as a receptor in intracellular signaling and controls a pathway that involves ERK1/ERK2- induced activation [9]. SDC4-ROS1 fusion proteins have activated kinase properties [2]. Recently, it has been demonstrated that crizotinib, a small molecule which is approved for treatment of ALK-positive lung cancer, has additional activity in lung adenocarcinoma with ROS1 translocations [3]. In this report, we describe a young male patient with novel SDC4-ROS1 fusion variant (E5:E34) pleural metastatic lung adenocarcinoma who experienced significant tumor shrinkage under crizotinib treatment.

Several methodological approaches for searching for ROS1 fusions have been developed and reported in the literature [2]. For the detection of ROS1 translocations, FISH appears to be best suitable since this method provides data within only 2 days after initial diagnosis. Quantitative real-time reverse transcriptase PCR (qRT-PCR) also could being used for detect the ROS1 translocations, however, which requires knowledge of all fusion variants for primer design but cannot detect previously unreported fusion partners. NGS has emerged as a powerful
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approach to look for genetic abnormalities. Whole-genome and whole-transcriptome sequencing has been used to identify ROS1 rearrangements. In this report, novel SDC4-ROS1 fusion variant (E5:E34) was identified by NGS technology. However, such approaches cannot readily be generalized to wide-scale clinical testing at present. Indeed, its clinical application demands high levels of accuracy, sensitivity and specificity. DNA- and RNA-sequencing remains expensive and the infrastructure, expertise in bioinformatics and time necessary to complete sequence analysis are still significant barriers.

The identification of molecular mechanisms conferring resistance to TyrosineKinase Inhibitor (TKIs) is a key-step to improve therapeutic results for patients with oncogene-addiction. Four ROS1 kinase domains mutations (S1986Y/F, G2032R and D2033N) have so far been reported as mechanism of crizotinib resistance in ROS1-rearranged NSCLC [10]. Besides ROS1 kinase domains mutations, crizotinib resistance has been reported asome due to EGFR, RAS, or KIT signaling activation [10, 11]. First (crizotinib), second (ceritinib, brigatinib) and third (lorlatinib (PF-0646392)) generation compounds are either approved or currently under evaluation in clinical trials. Currently this young patient disease is still stable with no cough or shortness of breath, however, we are always concerned about the possible occurrence of drug resistance at any time.

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Disclosure of conflict of interest

None.

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