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Case report

Targeted next-generation sequencing revealed distinct clinicopathologic and molecular features of *VCL-ALK* RCC: A unique case from an older patient without clinical evidence of sickle cell trait

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ABSTRACT

Anaplastic lymphoma kinase (*ALK*)-rearranged renal cell carcinoma (RCC) is a novel entity of rare tumors with only 10 cases reported in the literature. Three RCC cases bearing *VCL-ALK* gene fusion were all young African American patients and associated with sickle cell trait notably. In contrast to the 3 reported cases, this neoplasm occurred in a middle-age woman (57 years old) without any evidence of sickle cell trait and demonstrated an infiltrating growth pattern with tubular, tubulopapillary, and tubulocystic structures, overlapping with collecting duct carcinoma and renal medullary carcinoma. Abundant intraluminal mucin was also noted significantly in the histologic sections. Immunostaining showed strong membranous labeling for ALK protein. We applied a large panel-targeted next-generation sequencing to explore the molecular alterations in the current case, revealing a driver oncogene *VCL-ALK* gene fusion co-occurring with pathogenic mutations in *EP300* and *TRRAP* genes. Thereafter, fluorescence in situ hybridization assay was used to detect the *ALK* gene rearrangement. Reverse transcription polymerase chain reaction confirmed the presence of a *VCL-ALK* gene fusion, a fusion of *VCL* exon 16 to *ALK* exon 20. Our report draws the attention to the possibility that *VCL-ALK* genotype can be involved in older patients unassociated with sickle cell trait, also expanding the spectrum to *ALK*-rearranged RCC.

1. Introduction

Recent studies have indicated that a minority of renal cell carcinomas (RCCs) harbor recurrent gene rearrangements of the anaplastic lymphoma kinase gene (*ALK*) at 2p23 [1–7], the cytogenetic abnormalities observed more commonly in anaplastic large cell lymphoma [8], inflammatory myofibroblastic tumor [9,10], and pulmonary adenocarcinoma [11–13]. Since the first case series of *ALK*-rearranged RCC dates back to 2011, only 10 cases have been reported in the literature, involving *VCL-ALK* gene fusion (3 cases), *TPM3-ALK* gene fusion (2 cases), and *EML4-ALK* gene fusion (2 cases). Given the limited number of *ALK*-rearranged RCC, it is not currently included in the 2016 World Health organization (WHO) renal tumor classification, but recognized as a provisional new entity in the 2013 ISUP (International Society of Urological Pathology) Vancouver Classification of renal tumor. [14]

To our knowledge, three of the *ALK*-rearranged RCCs involving *VCL* gene fusion partner, were all young African American patients and associated with sickle cell trait notably [1,2,6]. In addition, *VCL-ALK* RCCs also share similar distinctive morphologic and immunohistochemical features, including solid architecture, distinctive polygonal to spindle-shaped cells with prominent intracytoplasmic lumina, prominent lymphoplasmacytic infiltrate, intact INI1 protein, and a low Ki-67 index. Accordingly, it has been proposed that *VCL-ALK* RCCs possibly represent a distinctive entity and may be a distinctive complication of sickle cell trait [6,15], but the overall numbers remain too limited to draw more definitive conclusions.

We present herein a unique case of an RCC with *VCL-ALK* gene fusion identified by large panel-targeted next-generation sequencing. Unlike the previously 3 cases reported in the literature, this case occurred in a middle-aged Chinese woman lacking the evidence of sickle cell trait or the related hemoglobinopathies.

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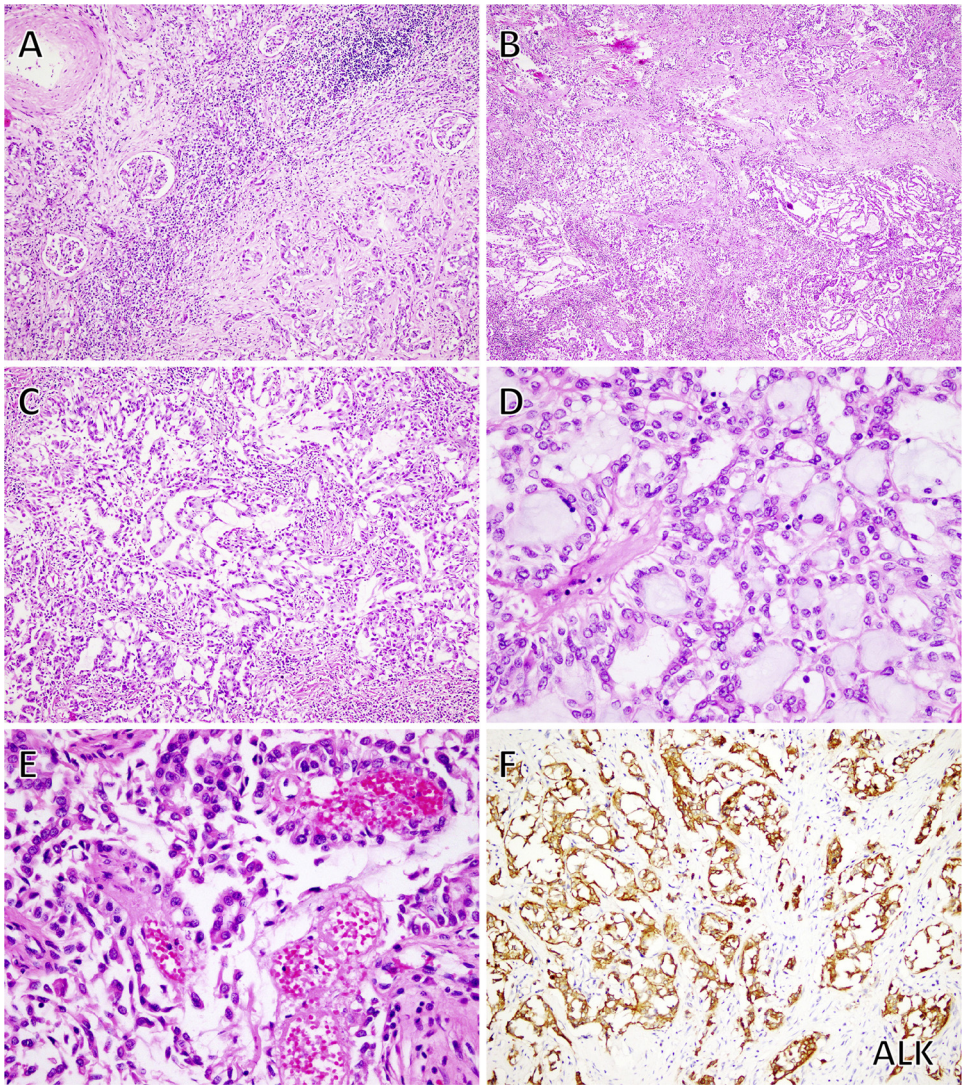

Fig. 1. A and B, The neoplasm demonstrated an infiltrating growth pattern between tubules and glomeruli with a desmoplastic and inflammatory stroma. C and D, The feature was a tubular, tubulopapillary, or tubulocystic tumour, especially with apparent intraluminal mucin. E, Normal erythroid cells instead of sickled erythrocytes were observed in the histologic sections. F, The tumor cells showed diffuse and strong positive for ALK protein.

2. Materials and methods

2.1. Histological and immunohistochemical analysis

The tumorectomy specimen preparation followed standard pathological and immunohistochemical procedures. Tumor tissues were fixed in 10% formalin and paraffin-embedded (FFPE). Sections 3 μm thick were immunohistochemically stained using antibodies against the following: ALK (OTI1H7, ready to use, OriGene, USA); INI1 (25/BAF47, 1:100; BD Transduction Laboratories), FH(J-13, 1:1000; Santa Cruz, CA), TFE3 (SC-5958, 1:300; Santa Cruz, CA), TFEB (ab2636, 1:300; Abcam, Cambridge, UK), PAX8 (4H7B3, 1:100; ProteinTech Group, Rosemont, IL), CD10 (56C6, 1:100; Novocastra, Milton keynes, UK), CK7 (OV-TL12/30, 1:300; Zymed, Grand Island, NY), E-cadherin (MAB-0589, ready to use, Maixin Biotechnology Ltd), and Ki-67 (MIB-1, 1:300, Maixin Biotechnology Ltd). Hematoxylin and eosin and immunohistochemical staining analyses were reviewed by two experienced pathologists (R.Q. and X.Q.Y). The clinicopathological features and available follow-up information were obtained.

2.2. Molecular genetic studies

2.2.1. Targeted next-generation sequencing

Next-generation sequencing (NGS) for a panel designed to target 520 cancer-specific genes was performed by Burning Rock Clinical Lab Co., Ltd (Guangzhou, China) on Illumina NextSeq™ 500 paired-end system. All procedures were conducted according to the manufacturers' protocols. Briefly, DNA was extracted from tumor FFPE tissues using a QIAamp Mini kit (Qiagen Inc., USA). For DNA library preparation, using the M220 Focused-ultrasonicator (Covaris, Woburn, MA, USA), genomic DNA was sheared into fragments (range of 200-400bp) followed by end-repairing, phosphorylation, and adaptor ligation, and then the fragments were size selected by Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA), followed by PCR amplification and hybrid selection with magnetic beads. The sequencing data were mapped to the human reference sequence hg19 using Burrows-Wheeler Aligner software (BWA).

2.3. Detection of ALK gene rearrangement by FISH

Fluorescence in situ hybridization (FISH) analysis was conducted on formalin-fixed, paraffin embedded tissue sections as previously

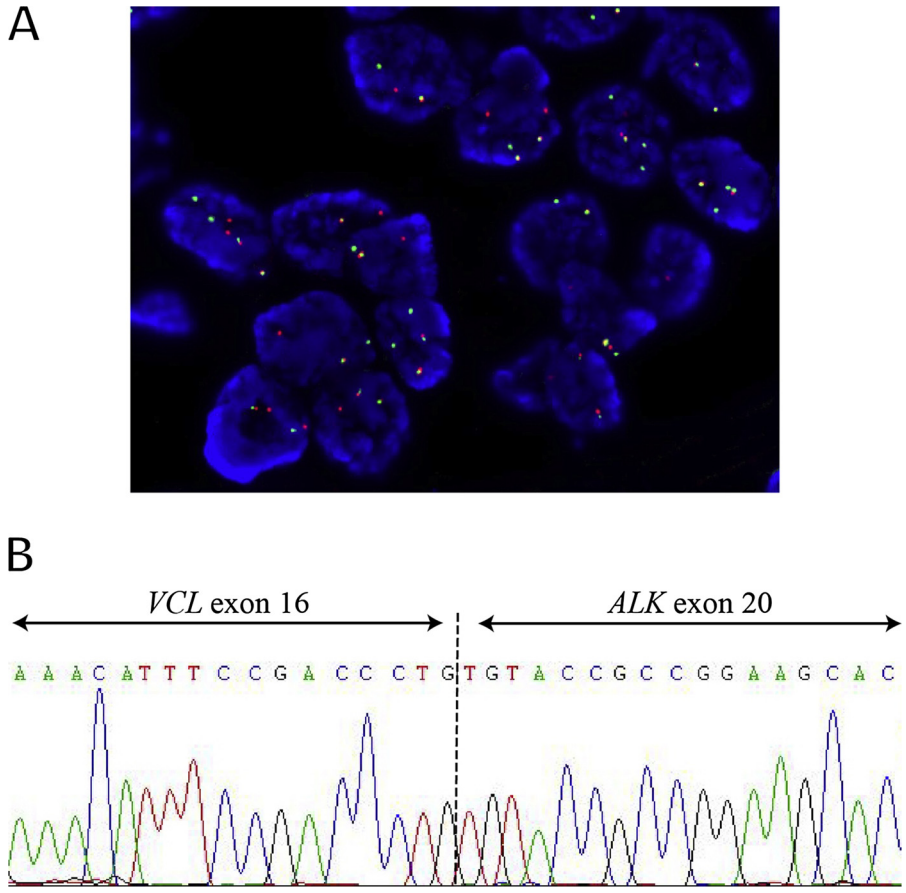

Fig. 2. A, FISH analysis using an *ALK* dual-color break-apart probe demonstrated split red and green signals indicative of a rearrangement of the *ALK* gene. B, Sequencing of the RT-PCR products confirmed a fusion of *VCL* exon 16 with *ALK* exon 20.

described. For the detection of *ALK* gene rearrangement, we employed a dual-color, *ALK* break-apart probe (Vysis, Downers Grove, IL). The signals were considered to be split when the green and red signals were separated by a distance ≥2 signal diameters. 200 interphase tumor nuclei were examined for probe signals by fluorescence microscopy at ×1000 magnification. To avoid false-positive interpretations resulting from nuclear truncation, only nonoverlapping tumor nuclei were evaluated. If > 50% of nuclei showed a split FISH pattern, it was considered as a positive result.

2.4. Reverse transcription polymerase chain reaction and Sanger sequencing

To confirm the presence of the *VCL-ALK* fusion transcript, reverse transcription polymerase chain reaction (RT-PCR) was performed. The following primers were designed to amplify a predicted 139-bp product: *VCL* exon 16 (F): GCTGTGGCTGGAAACATT, *ALK* exon 20 (R): TGGG GTTGTAGTCGGTCAT. For sequence analysis, the PCR products were purified using the Wizard PCR Preps Purification System (Promega Corp., Madison, WI, USA), and sequencing was performed using Big Dye Terminator and an ABI Basecaller (Applied Biosystems, Carlsbad, CA, USA).

3. Results

3.1. Clinicopathologic findings

The patient is a 57-year-old Chinese woman without clinical evidence of sickle cell trait or other significant clinical symptoms. However, hemoglobin electrophoresis was absent, which was a more definitively examination to exclude sickle cell trait. Abdominal computed tomography (CT) scan revealed a solid mass with a diameter of 5.5 cm in the upper pole of the left kidney, for which the patient underwent a left radical nephrectomy. The patient received no post-operative chemotherapy, and had a poor clinical course, progressing to death from disease at 20 months after surgery.

On microscopy, there was marked overlap with features seen in collecting duct carcinoma (CDC) and renal medullary carcinoma (RMC), including tubular, tubulopapillary, and tubulocystic structures with an infiltrating growth pattern between tubules and glomeruli in a desmoplastic and inflammatory stroma (Fig. 1A, B, and C). At intermediate power, abundant intraluminal mucin was also noted significantly (Fig. 1D), which was more frequently present in RMC. The neoplastic cells exhibited pale eosinophilic or clear cytoplasm and distinct cell membranes. The nuclei were large, vesicular, and moderately pleomorphic and contain variably prominent nucleoli. Sickled erythrocytes were absent within the tumor and in the adjacent renal tissue (Fig. 1E). Original assessment of this tumor included negative immunostains for TFE3 and TFEB, focally positive for PAX8 and CD10, and strongly positive for CK7 and E-cadherin. FH and INI1 staining were both retained completely. Additionally performed ALK decorated the majority of cells with strong expression (Fig. 1F). The nuclear proliferative index (Ki-67) was estimated as 10%.

3.2. Molecular genetic findings

3.2.1. NGS results

The sample had high DNA quality with expected depth of coverage and low PCR duplication rate. Targeted NGS assay revealed a rare driver oncogene, *VCL-ALK* gene fusion (*VCL* intron 16-*ALK* intron 19, supplementary material 1). Notably, pathogenic alterations in the

tumor specimen also included *EP300* splice donor mutation (c.3671 + 1G > A, VAF = 3.16%) and *TRRAP* nonsense mutation (p.R1719*, VAF = 2.01%) (supplementary material 2 and 3). Other variants of uncertain pathogenicity were detected including *IDH2*, *ANKRD11*, *CDK12*, *DNMT1*, *MSH3*, *PRKN*, *KMT2C*, and *NOTCH1* missense mutations. The tumor mutation burden (TMB) obtained from the targeted sequencing was 7.94 mutations per megabase (Muts/Mb), and no copy number variation (CNV) was identified.

3.3. FISH and Sanger sequencing results

Interphase FISH analysis demonstrated that more than 50% of tumor nuclei showed split *ALK* signals, one fused and one split red-green signal pattern (Fig. 2A). The presence of *VCL-ALK* gene fusion was then corroborated by RT-PCR using specific primers. Sanger sequencing of the RT-PCR product verified an aberrant transcript composed of *VCL* exon 16 fused to *ALK* exon 20 (Fig. 2B).

4. Discussion

The initial description of *VCL-ALK* RCC occurring in a 16-year-old boy was presented by Debelenko et al in 2011, [1] and shortly thereafter, in close correspondence with the case from Marino et al in a 6-year-old boy [2]. These early reports were followed by the study of Smith et al in 2014 [6], who reported the third similar case harboring a specific *VCL-ALK* gene fusion. Importantly, all reportedly cases occurred in young African-American patients, and were noted a strong association with sickle cell trait. Histological and immunohistochemical examination of these 3 cases showed similar distinctive features, [6] such as sheets of polygonal to spindle-shaped cells with large vesicular nuclei, abundant eosinophilic cytoplasm, frequent intracytoplasmic lumina, intact INI-1 protein, and low Ki-67 index. None of the patients with *VCL-ALK* RCC followed an aggressive course, showing well and disease free at 4 months, 21 months, and 16 months after surgery, respectively. As the other 7 cases of *ALK*-rearranged RCCs, including 2 *TPM3-ALK*, 2 *EML4-ALK*, and 3 with unknown partner showed significant heterogeneity in clinical and morphological features, the definitive association of the genotypes with clinicopathologic features could not be identified. [3–5,7]

Notably, the results of the present study were different from these earlier observations. The specific *VCL-ALK* gene fusion was identified in our case using the targeted next-generation sequencing, which was instead from an older female, aging 57 years at surgery and without any history of sickle cell trait. In contrast with the distinctive features of *VCL-ALK* RCC described in the literature, this case was associated microscopically with high-grade histologic features and showed highly infiltrative growth pattern with tubules, tubulopapillary, and tubulocystic structures, indistinguishable from CDC and RMC. More specifically, abundant intraluminal mucin, preciously undescribed in *VCL-ALK* RCC, was apparent in our case. There was no evidence of sickled erythrocytes in the histologic sections. In addition, the patient displayed clinically aggressive behavior, and died of disease 20 months later.

The diagnostic classification of *ALK*-rearranged RCC remains challenging due to their rarity, variable histologic features and often non-specific immunophenotype. The detection of specific genetic features is critical from both diagnostic and therapeutic standpoints. In this study, we applied a targeted next-generation sequencing of 520 cancer-related genes to explore the molecular pathogenesis, revealing the *ALK* gene fused to the *VCL* gene. Not only could this genetic alteration be considered as a reliable molecular diagnostic marker, but has potential therapeutic implications as well. An ALK fusion protein inhibitor crizotinib has been used extensively in the treatment of *ALK*-rearranged non-small cell lung cancer, [16–18] and also has shown to be an effective therapy in other multiple tumor types including inflammatory myofibroblastic tumor [19] and diffuse large B cell lymphoma [20]. Patients with *ALK*-rearranged RCC may be also benefit from *ALK* inhibitor therapy.

Beyond the specific *VCL-ALK* gene fusion with significant oncogenic activity, mutations in *EP300* gene and *TRRAP* gene have also been implicated in the genesis of this case. *EP300*, which encodes the adenovirus E1A-associated cellular p300 transcriptional co-activator protein, functions as histone acetyltransferase that regulates gene expression by acetylating chromatin and is important in the processes of cell proliferation and differentiation. [21,22] Defects in *EP300* may play a role in epithelial cancer, such as lung cancer, breast cancer, prostate cancer etc. [23–25] *TRRAP* (transformation/transcription domain-associated protein) is also a component of histone acetyltransferase complex and regulates gene expression and embryonic development. [26,27] Deregulation of *TRRAP* may have some effects in several types of cancer including glioblastoma multiforme. [28] Although splice donor mutation in *EP300* and nonsense mutation in *TRRAP* were observed in this case, they co-occurred with the presence of *VCL-ALK* gene fusion making it difficult to verify the exact role of *EP300* and *TRRAP* mutations in the development of this case.

In conclusion, for the first time, we report a unique RCC case bearing a fusion of *VCL* and *ALK* genes, but occurring in a middle-age female and without any history of sickle cell trait nor sickled red erythrocytes noted in the histologic sections. This is in contrast to all previously described *VCL-ALK* RCCs. Our report draws the attention to the possibility that *VCL-ALK* genotype can be involved in older patients unassociated with sickle cell trait, also expanding the spectrum to *ALK*-rearranged RCC. The targeted NGS is useful to confirm the diagnosis and to select patient for the targeted strategy with the ALK inhibitor.

Declaration of Competing Interest

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152651.

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