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Letters to the Editor

Distinct ALK-rearranged and VCL-negative papillary renal cell carcinoma variant in two adults without sickle cell trait

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To the Editor: A previous study by our group applied a dual-color break-apart *ALK* FISH probe set to a tissue microarray consisting of 334 clear cell renal cell carcinoma and 313 papillary renal cell carcinoma samples from adults consecutively treated by nephrectomy.[1] *ALK* rearrangements were rare, occurring in only two papillary renal cell carcinoma cases, which is <1% of all tumors studied. *ALK* rearrangement is demonstrated in column one of the Supplemental Figure S1 by the 1R1G1F pattern corresponding to split of one *ALK* gene (1R1G) and one intact *ALK* gene (1F). This pattern was confirmed to be uniform throughout each of the tumors by subsequent FISH on whole-cut sections. ALK protein overexpression was also confirmed by immunohistochemistry. These cases were both from older males, ages 61 and 59 years at surgery, without any history of sickle cell trait. Both patients with *ALK* rearrangement had a poor clinical course, surviving less than 5 years post diagnosis (4 years and 1.4 years to death from renal cell carcinoma).

A recent report by Debelenko *et al*[2] used FISH to confirm rearrangement of *ALK* at 2p23 in a renal tumor with a complex karyotype from a 16-year-old African American male with sickle cell trait. The partner of *ALK* was demonstrated to be *VCL* by 5′-rapid amplification of cDNA ends analysis and confirmatory RT-PCR and sequencing. Immunohistochemistry showed ALK protein expression in the tumor. At the time of surgery, this patient had a small regional lymph node metastasis and extensive primary tumor capsule infiltration, but was alive without disease recurrence through 4 months of post surgery follow-up. Concurrently, Marino-Enriquez *et al*[3] identified the exact same *VCL*/*ALK* fusion breakpoints using dual-color break-apart *ALK* FISH, RT-PCR and sequencing in a second renal carcinoma with t(2;10)(p23;q22). The tumor from this 6-year-old African American male with sickle cell trait was also positive by immunohistochemistry for ALK protein. Following tumor removal by radical nephroureterectomy with lymph node dissection, the child remained in remission through 21 months post surgery follow-up, despite the initial finding of vascular and renal sinus invasion.

In the present two ALK-rearranged renal cell carcinoma cases, FISH for VCL/*ALK* fusion on whole sections cut from paraffin-embedded tumor blocks was performed using a nearly identical and overlapping dual-color, dual-fusion probe set to that described by Debelenko *et al*[2] (RP11-836A7, CTD-2025L2, RP11-664B23, and RP11-610M22 spanning *VCL* in SpectrumOrange and RP11-1062I24, CTD-2245E6, RP11-328L16, RP11-983I21, CTD-2544I11 and RP11-684O3 spanning *ALK* in SpectrumGreen). The specificity and signal quality of each clone was confirmed by hybridization to normal blood metaphases before being combined into a probe set. FISH patterns were established by scoring 200 interphase tumor nuclei. The results in column two of the Supplemental Figure S1 shows two *VCL* signals and three *ALK* signals (2R3G) without any yellow fusion signal in both cases (39.5% in patient 1 and 42% in patient 2), indicating *ALK* is rearranged but not fused to *VCL*. Specifically, one green signal is an intact *ALK* gene, and the other two green signals result from disruption of the green probe that covers *ALK*, with a portion remaining on chromosome 2 and a portion moving to another area of the genome but not fusing to *VCL*. Less than 100% of cells scored have the 2R3G abnormal pattern due to the presence of normal cells in the tissue combined with the technical artifact of using cut tissue sections which decreases the proportion of intact nuclei.

Debelenko *et al*[2] also reported their case was *TFE3* positive by immunohistochemistry, although the karyotype did not show Xp11.2 rearrangement and FISH for *TFE3* was not attempted. Both patients in our series are negative for *TFE3* rearrangement as determined by FISH with a custom-designed break-apart probe set. The result of scoring 200 interphase nuclei in each case was a 1F1A pattern (98% in patient 1 and 97% in patient 2), shown in the third column of the Supplemental Figure S1. This is consistent with a single X chromosome centromere and one intact *TFE3* gene, the expected pattern for a normal male.

As demonstrated in the fourth column of the Supplemental Figure S1 and described in Sukov *et al*,[1] both *ALK*-rearranged but *VCL*- and *TFE3*-negative tumors had similar and distinctive histological features. A papillary architecture was noted in which the papillae contained foamy macrophages and were lined by cells with clear to eosinophilic cytoplasm and dense nuclei with irregular contours and inconspicuous nucleoli without psammoma bodies. The tumor from patient 2 also had coagulative necrosis and regions of solid growth in which the tumor cells had primary eosinophilic

cytoplasm and larger nuclei with more prominent nucleoli.

Such findings are in contrast to those reported by both cases with *VCL*/*ALK* fusion. The right kidney tumor from Marino-Enriquez *et al*[3] had a focal reticular growth pattern, prominent lymphoplasmacytic infiltrate, stromal desmoplasia and solid sheet of polygonal-to-spindle cells with vesicular nuclei and abundant eosinophilic cytoplasm with intracytoplasmic lumina. The immunohistochemistry profile included expression of EMA and the cytokeratins MNF116, AE1/AE3 and CAM5.2, as well as maintaining nuclear INI-1 expression. The final pathological diagnosis was renal medullary carcinoma (RMC). In the study by Debelenko *et al*,[2] the initial needle-core biopsy finding was RMC of sickle cell trait. Analysis of a nephrectomy specimen 5 months later was described as having solid and diffuse sheet-like growth of large polygonal-to-round tumor cells with abundant and vaguely granular eosinophilic cytoplasm characterized by intracytoplasmic lumina and moderately pleomorphic clear nuclei and small nucleoli, occasional grooves and rare vacuoles. Immunohistochemistry demonstrated tumor cell expression of EMA, the cytokeratins AE1/AE3, CAM5.2 and CK7, as well as INI-1 expression, whereas CD10, renal antigen, S-100, HMB45 and WT1 were negative. The final pathological diagnosis was renal cell carcinoma of indeterminate subtype.

It is important to note that RMC often demonstrates significant variation in the microscopic appearance. Considering this, combined with the overlap in patient demographics, pathological features and especially the new genetic results from these two published cases, it suggests that the tumor from the Debelenko *et al*[2] study may be within the RMC spectrum.

In summary, *ALK* rearrangement in papillary renal cell carcinoma cases in older adults may represent a distinct pathological entity with poorer outcome. Our cases did not have *VCL* as a partner and *TFE3* was not disrupted. This is in contrast to all previously described categories, including kidney tumors with *VCL*/*ALK* fusion in young black males with sickle cell trait. Such distinction of *ALK*-rearranged pathological entities is of particular importance because of the potential benefit from *ALK* inhibitor therapy in these patients.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)

Reply to 'Distinct ALK-rearranged and VCL-negative papillary renal cell carcinoma variants in two adults without sickle cell trait'

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To the editor: ALK activation by chromosomal rearrangement in renal cell carcinoma (RCC) was first demonstrated by two independent pediatric pathology studies 2 years ago.[1,2] These reports coincided with the successful results of the clinical trial that tested targeted therapy with crizotinib in ALK-positive lung cancer.[3] Consequently, two recently published large series of adult RCCs cumulatively identified four new *ALK*-rearranged tumors.[4,5] Current data, combined from four independent studies during 2010–2012, include six ALK-rearranged tumors in a representative cohort of 884 RCCs of main morphologies arising in pediatric and adult patients of diverse ethnicities (Table 1).