

Case Report

Renal cell carcinomas with t(6;11)(p21;q12) presenting with tubulocystic renal cell carcinoma-like features

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Abstract: In this study, we reported an additional genetically confirmed case of renal cell carcinomas (RCCs) with t(6;11)(p21;q12) showing an unusual histological pattern. Histologically, the tumor was entirely composed of small to intermediate sized tubules and cysts. The tubules and cysts were lined by a single layer of flat, hobnail, cuboidal to columnar epithelial cells. Most cells demonstrated abundant eosinophilic cytoplasm with regular, round or oval nuclei and some inconspicuous nucleoli. All these morphological features are suggestive of tubulocystic carcinoma of the kidney. However, the tumor demonstrated moderately (2+) or strongly (3+) positive staining for TFEB, Cathepsin K, Ksp-cadherin, and vimentin but negative for TFE3, CD10, HMB45, melan A, CKpan, and CK7. Using a recently developed TFEB split FISH assay, the presence of TFEB rearrangement was demonstrated. Our results support the clinical application of a TFEB break-apart FISH assay for diagnosis and confirmation of TFEB RCC and further expand the morphologic spectrum that may be present in these neoplasms, sometimes raising a challenging differential diagnosis with other renal tumors.

Keywords: Kidney, translocation, renal carcinomas with t(6;11)(p21;q12), TFEB, cathepsin K, fluorescence in situ hybridization (FISH), molecular genetics, differential diagnosis

Introduction

Renal cell carcinomas (RCCs) with t(6;11)(p21;q12) are very uncommon neoplasms arising more commonly in children and young adults, and characterized by specific chromosome translocation, involving the transcription factor EB (TFEB) [1]. To our knowledge, fewer than 50 cases have been described in the literature [1-14]. Morphologically, distinctive features of TFEB RCCs include nests of epithelioid cell morphology with clear cytoplasm, mimicking a typical clear cell RCC, and clusters of small cells, surrounding collagenous stroma formed by hyaline material, which we called "pseudorosettes" [4]. However, there has been increasing evidence that TFEB RCCs may demonstrate unusual morphologic features [10, 13, 14]. The full spectrum of morphologic features is not entirely elucidated and continues to expand. In this study, we reported an additional genetically confirmed case of TFEB RCCs showing an unusual histological pattern that highlight the challenging differential diagnosis of this tumor entity.

Case report

A 68-year-old man with no significant past medical history presented with 6 months history of intermittent lumbar pain. Abdominal computed tomography (CT) scan and ultrasound demonstrated a 2.5×2 cm sized mass in the right renal pelvis. A total nephrectomy was performed without chemotherapy or radiation therapy after surgery. Currently, the patient is well and no recurrence was observed with 23 months of follow-up.

Materials and methods

Light microscopy

Tissues were fixed in 10% formalin and embedded in paraffin. Sections of 3 mm thickness were stained with hematoxylin and eosin (HE), and immunohistochemistry. The following antibodies were used: TFEB (ab2636, Abcam, 1:300), TFE3 (SC-5958, Santa Cruz, 1:300), cathepsin K (3F9, Abcam, 1:300), Ksp-cadherin (4H6/F9, Zymed, 1:200), HMB45 (HMB45,

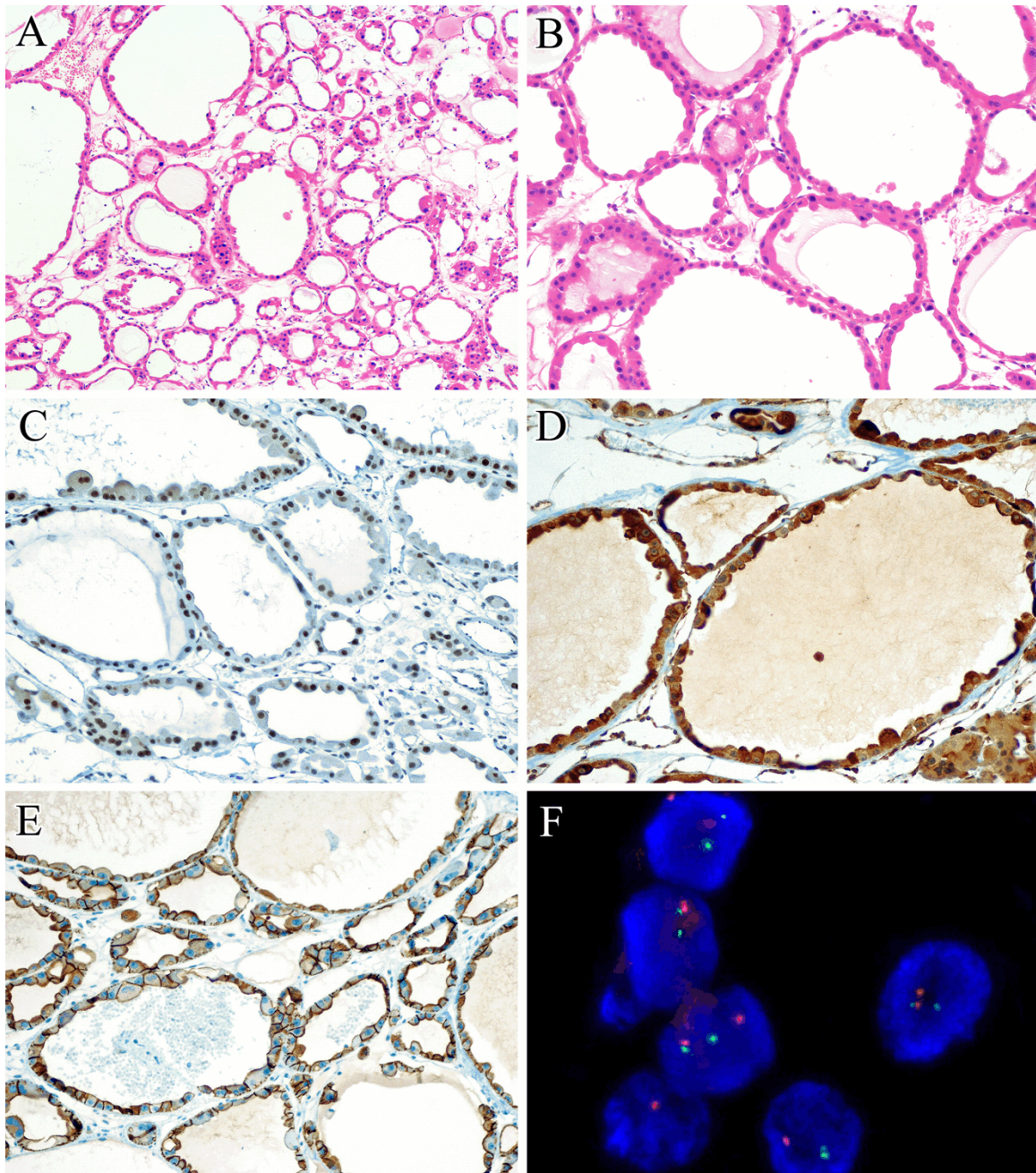


Figure 1. A. The tumor was entirely composed of small to intermediate sized tubules and cysts. They were separated by fibrous septa or hyaline stroma without an ovarian-type of stroma or desmoplastic reaction. (original magnification, $\times 100$). B. The tubules and cysts were lined by a single layer of flat, hobnail, cuboidal to columnar epithelial cells. Most cells demonstrated abundant eosinophilic cytoplasm with regular, round or oval nuclei and some inconspicuous nucleoli. All these morphological features are suggestive of tubulocystic carcinoma of the kidney (original magnification, $\times 200$). The tumor moderately to strongly expresses TFEB (C), cathepsin K (D), and Ksp-cadherin (E) (original magnification, $\times 200$). F. The split FISH assay results in normal combined hybridization signals and one pair of split signals in the case. FISH indicates fluorescence in situ hybridization. Nuclear staining with DAPI (blue).

Dako, 1:500), melan A (A103/M2-72, Neomarkers, 1:100), CD10 (56C6, Novocastra, 1:100), CKpan (AE1/AE3, Zymed, 1:150),

vimentin (V9, Zymed, 1:200), and CK7 (OV-TL12/30, Zymed, 1:300). Immunoreaction was performed using the labeled streptavidin-biotin

method with overnight incubation and Diaminobenzidine (3, 3'-diaminobenzidine) was used for visualization.

Immunoreactivity was interpreted in a semi-quantitative way assessing both staining intensity and percentage of positive cells, as described previously [15, 16]. For all antibodies, the resulting score was calculated by multiplying the staining intensity (0 = no staining, 1 = mild staining, 2 = moderate staining and 3 = strong staining) by the percentage of immunoreactive tumour cells (0–100). The immunostaining was considered 0 or negative when the score was <25; 1+ or weak, 26–100; 2+ or moderate, 101–200; and 3+ or strong, 201–300.

Detection of alpha-TFEB fusion by DNA PCR

Genomic DNA was extracted from the FFPE tissue samples of the tumor by the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Alpha-TFEB genomic junction was performed as recently described [2-4].

For sequence analysis, the PCR products were purified using the Wizard PCR Preps Purification System (Promega Corp.). Sequencing was performed using Big Dye Terminator and an ABI Basecaller (Applied Biosystems).

TFEB fluorescence in situ hybridization (FISH)

As previous study, a split FISH ('break-apart') assay with probes centromeric (Green 5-fluorescein dUTP) and telomeric (Red 5-ROX dUTP) for TFEB was conducted to determine if a TFEB gene rearrangement was present [13]. The normal result is a combination (green and red) signal, whereas TFEB fusion results in a split signal. Signals were considered to be split when the green and red signals were separated by a distance >2 signal diameters. A positive result included 1 fused or closely approximated green-red signal pair (representing the uninvolved copy of the 6 chromosome) and an additional pair of split signals. A minimum of 100 tumor cell nuclei were examined under fluorescence microscopy at ×1000 magnification. Only nonoverlapping tumor nuclei were evaluated. Based on other commercially available break-apart FISH assays and TFEB break-apart FISH assays, a positive result was reported

when >10% of the tumor nuclei showed the split-signal pattern [13, 17-19].

Results

Morphology

Histologically, the tumor was entirely composed of small to intermediate sized tubules and cysts. They were separated by fibrous septa or hyaline stroma without an ovarian-type of stroma or desmoplastic reaction. The tubules and cysts were lined by a single layer of flat, hobnail, cuboidal to columnar epithelial cells. Most cells demonstrated abundant eosinophilic cytoplasm with regular, round or oval nuclei and some inconspicuous nucleoli. Neither clear cell areas nor papillary patterns were identified in any areas of the tumor. All these morphological features are suggestive of tubulocystic carcinoma of the kidney (**Figure 1A** and **1B**).

Immunohistochemistry

The tumor demonstrated moderately (2+) or strongly (3+) positive staining for TFEB, Cathepsin K, Ksp-cadherin, and vimentin but negative for TFE3, CD10, HMB45, melan A, CKpan, and CK7. The presence of Ki-67 protein demonstrated a low proliferation rate, with few Ki-67-positive nuclei (**Figure 1C-E**).

Molecular analysis

None of the Alpha-TFEB fusion genes was identified in the tumor.

TFEB FISH analysis

In this case, the split FISH assay demonstrated the presence of TFEB rearrangement associated with 6p21 translocation (**Figure 1F**).

Discussion

In this study, we reported an additional genetically confirmed case of TFEB RCCs showing an unusual histological pattern. Histologically, the tumor was entirely composed of small to intermediate sized tubules and cysts. They were separated by fibrous septa or hyaline stroma without an ovarian-type of stroma or desmoplastic reaction. The tubules and cysts were lined by a single layer of flat, hobnail, cuboidal to columnar epithelial cells. Most cells demonstrated abundant eosinophilic cytoplasm with

regular, round or oval nuclei and some inconspicuous nucleoli. Neither clear cell areas nor papillary patterns were identified in any areas of the tumor. All these morphological features are suggestive of tubulocystic carcinoma of the kidney. However, the tumor demonstrated moderately (2+) or strongly (3+) positive staining for TFEB, Cathepsin K, Ksp-cadherin, and vimentin but negative for TFE3, CD10, HMB45, melan A, CKpan, and CK7. Using a recently developed TFEB split FISH assay, the presence of TFEB rearrangement was demonstrated. Based on the results of immunohistochemical examination and molecular genetic finding, this case was classified as Renal cell carcinomas (RCCs) with t(6;11)(p21;q12).

When reviewing published data with histopathologic description, TFEB RCCs are characteristically considered to exhibit certain histologic features, including large epithelioid cells with voluminous clear to slightly eosinophilic cytoplasm and clusters of small cells, usually clustered around hyaline material, which we called pseudorosettes [4, 13]. However, unusual morphologies mimicking other RCC subtypes have also been reported, including tumors resembling chromophobe cell RCC, clear cell RCC, papillary, tubular, oncocytic papillary morphology, oncocytoma and epithelioid angiomyolipoma structures [4, 9-14, 20, 21]. To our knowledge, the morphologic pattern mimicking tubulocystic RCC has not been previously reported in these tumors. These unusual features have further expanded the morphologic spectrum to be found in TFEB RCCs.

Considering the diversity of histologic findings that TFEB RCCs may exhibit, diagnosis should be based not only on morphology itself but also on immunophenotypic and molecular genetic findings. As the translocations present in these tumors lead to overexpression of the TFEB protein, detection of TFEB protein overexpression by immunohistochemistry is currently the most commonly used diagnostic technique in clinical practice [4]. Cathepsin K is a cysteine protease from the papain family, which plays an important role in osteoclast function. Expression of cathepsin K in osteoclasts is regulated by MITF [22]. As recent studies have demonstrated cathepsin K to be a transcriptional target of the microphthalmia-associated transcription factor family, immunohistochemistry antibody to cathepsin K has been utilized in the diagnosis

of microphthalmia-associated transcription factor/transcription factor E (MITF/TFE) family renal translocation tumors [16, 22-24]. Aside from TFEB and cathepsin K, a recently developed antibody of Ksp-cadherin, which was identified as a specific marker for renal neoplasms of distal nephron derivation/differentiation such as chromophobe RCC and oncocytoma has been shown to be a highly sensitive and relatively specific marker for TFEB RCCs and provided support for a distal nephron origin of these renal tumors [13, 25]. In this study, the tumor showed moderate to strong immunoreactivity for TFEB, Cathepsin K, and Ksp-cadherin, in keeping with the findings of previous studies.

Although the morphology and immunophenotype of most TFEB RCCs are so distinctive that it can be diagnosed only from standard hematoxylin and eosin staining and immunohistochemistry, molecular pathology methods such as PCR, RT-PCR, and FISH are still gold standard methods for these tumors, especially those with unusual morphologic features. Cytogenetic karyotypic analysis and reverse transcriptase polymerase chain reaction (RT-PCR) are 2 common methodologies for identifying this translocation. Unfortunately, both methods are limited by the availability of tumor cells and fresh frozen tissue, which are not always easy to apply in routine surgical pathology practice. Furthermore, because of the scattered genomic breakpoints in large introns and diverse fusion patterns reported by previous studies, it is reasonable to conclude that the detection of gene fusion by PCR is still less reliable than other molecular methods due to their variable breakpoints and amplification of large size range [4, 5, 9, 12, 13]. The potential risk of false negatives and inefficient PCR amplifications will further complicate the analysis. Maybe for these reasons, none of the Alpha-TFEB fusion genes was identified in the current tumor using the previously designed PCR primers.

Interphase fluorescence in situ hybridization (FISH), performed on formalin-fixed, paraffin-embedded (FFPE) tissues, is a helpful method for assessment of gene fusion status in tumors associated with specific translocations, such as Ewing sarcoma, synovial sarcoma, and Xp11.2 RCC [18, 19, 26]. Recently, several TFEB break-apart FISH assays have been devel-

oped to detect TFEB gene rearrangements [2, 13, 14]. In the present study, we used a TFEB break-apart FISH assay to provide molecular confirmation of a diagnosis of TFEB RCC with an unusual morphology. TFEB break-apart FISH assay should be a useful complementary method for confirming the diagnosis of TFEB RCC.

In summary, we reported an additional genetically confirmed case of TFEB RCCs showing an unusual histological pattern. Our results support the clinical application of a TFEB break-apart FISH assay for diagnosis and confirmation of TFEB RCC and further expand the morphologic spectrum that may be present in these neoplasms, sometimes raising a challenging differential diagnosis with other renal tumors.

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Disclosure of conflict of interest

The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

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