HHS Public Access

Author manuscript

Am J Surg Pathol. Author manuscript; available in PMC 2017 June 01.

Published in final edited form as:

Am J Surg Pathol. 2016 June ; 40(6): 723–737. doi:10.1097/PAS.0000000000000631.

TFE3-Fusion Variant Analysis Defines Specific Clinicopathologic Associations Among Xp11 Translocation Cancers

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Abstract

Xp11 translocation cancers include Xp11 translocation renal cell carcinoma (RCC), Xp11 translocation perivascular epithelioid cell tumor (PEComa), and melanotic Xp11 translocation renal cancer. In Xp11 translocation cancers, oncogenic activation of TFE3 is driven by the fusion of *TFE3* with a number of different gene partners, however, the impact of individual fusion variant on specific clinicopathologic features of Xp11 translocation cancers has not been well defined. In this study, we analyze 60 Xp11 translocation cancers by fluorescence in situ hybridization (FISH) using custom BAC probes to establish their *TFE3* fusion gene partner. In 5 cases RNA sequencing (RNA-seq) was also used to further characterize the fusion transcripts. The 60 Xp11 translocation cancers included 47 Xp11 translocation RCC, 8 Xp11 translocation PEComas, and 5 melanotic Xp11 translocation renal cancers. A fusion partner was identified in 53/60 (88%) cases, including 18 *SFPQ (PSF),* 16 *PRCC*, 12 *ASPSCR1 (ASPL),* 6 *NONO*, and 1 *DVL2*. We provide the first morphologic description of the *NONO-TFE3* RCC, which frequently demonstrates sub-nuclear vacuoles leading to distinctive suprabasal nuclear palisading. Similar sub-nuclear vacuolization was also characteristic of *SFPQ-TFE3* RCC, creating overlapping features with clear cell papillary RCC. We also describe the first RCC with a *DVL2-TFE3* gene fusion, in addition to an extrarenal pigmented PEComa with a *NONO-TFE3* gene fusion. Furthermore, among neoplasms with the *SFPQ-TFE3, NONO-TFE3, DVL2-TFE3* and *ASPL-TFE3* gene fusions, the RCC are almost always PAX8-positive, cathepsin K-negative by immunohistochemistry, whereas the mesenchymal counterparts (Xp11 translocation PEComas, melanotic Xp11 translocation renal cancers, and alveolar soft part sarcoma) are PAX8-negative, cathepsin K-positive. These findings support the concept that despite an identical gene fusion, the RCCs are distinct from the corresponding mesenchymal neoplasms, perhaps due to the cellular context in which the translocation occurs. We corroborate prior data showing that the *PRCC-TFE3* RCC are the only known Xp11 translocation

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Disclosures: Supported in part by: P01CA47179 (CRA), P50 CA 140146-01 (CRA), Cycle for Survival (CRA), Kristin Ann Carr Foundation (CRA), Dahan Translocation Carcinoma Fund (PA)

RCC molecular subtype which is consistently cathepsin K positive. In summary, our data expand further the clinicopathologic features of cancers with specific *TFE3* gene fusions, and should allow for more meaningful clinicopathologic associations to be drawn.

Keywords

Renal Neoplasm; TFE3; Translocation

INTRODUCTION

Xp11 translocation renal cell carcinomas (RCC) were first officially recognized by the World Health Organization (WHO) in 2004, following the initial detailed morphologic descriptions that were published in 2001 and 2002 (1,2). Xp11 translocation RCC bear chromosome translocations that result in one of a variety of gene fusions that involve the *TFE3* transcription factor gene, which maps to the Xp11.2 locus. Reported *TFE3* fusion partners include *ASPSCR1 (ASPL), PRCC, SFPQ1 (PSF), NONO, CLTC, PARP14, LUC7L3,* and *KHSRP* (3,4,5,6). Xp11 translocation RCC comprise the majority of pediatric RCC and approximately 1–4% adult RCC (7–10). While a variety of morphologic patterns have been described (11), the most common appearance is that of an RCC with papillary architecture, clear cells, and psammoma bodies. By immunohistochemistry, these tumors frequently underexpress cytokeratins, but frequently express melanocytic markers and the cysteine protease cathepsin k, which distinguishes them from more common RCC subtypes (12–14). Overall, outcome is similar to that of clear cell RCC, with increased age and advanced stage being poor prognostic factors (15,16). While immunohistochemistry for overexpressed TFE3 fusion proteins was initially the only method to confirm this diagnosis in formalin-fixed, paraffin embedded archival material (17), break-apart fluorescence in situ hybridization (FISH) demonstrating *TFE3* gene rearrangement is now the preferred method (10, 11,18,19). However, *TFE3* break-apart FISH does not provide information as to the specific fusion partners of *TFE3*. In fact, data on the clinicopathologic features of the subtypes of Xp11 translocation RCC associated with specific fusion partners is limited, as demonstration of the fusion partner has typically required fresh tissue for either cytogenetics or reverse transcriptase polymerase chain reaction (RT-PCR) assays.

Two other Xp11 translocation cancers which morphologically overlap with the Xp11 translocation RCC include Xp11 translocation PEComas and melanotic Xp11 translocation renal cancers. Xp11 translocation PEComas differ from typical PEComas in that they typically affect younger patients, have purely or predominantly epithelioid clear cell morphology, are not associated with Tuberous Sclerosis syndrome, do not express muscle markers by immunohistochemistry, and are not associated with *TSC2* gene alterations (20–24). The most commonly identified fusion in Xp11 translocation PEComas has been *SFPQ-TFE3*, with a rare case demonstrating a *DVL2-TFE3* gene fusion (22). Melanotic Xp11 translocation renal cancers were initially described before the Xp11 PEComas, and have been thought to overlap most with PEComa, though their renal origin raised the possibility of their representing Xp11 translocation RCC that do not express renal tubular markers (24–26). Significant morphologic overlap between Xp11 translocation RCC, Xp11 translocation

PEComa, and melanotic Xp11 translocation renal cancers has been described. In the few cases of melanotic Xp11 translocation renal cancer in which a specific fusion has been identified, the gene fusion has always been *SFPQ-TFE3* (24,26).

The development of FISH probes for the commonly identified *TFE3* fusion partners allows subtyping of Xp11 translocation neoplasms in archival material, vastly increasing the number of cases that can be analyzed. Subtyping of Xp11 translocation-associated cancers should allow more meaningful clinicopathologic associations to be drawn, such as the differences previously described in a review of the published literature between the *ASPSCR1-TFE3* RCC and the *PRCC-TFE3* RCC (16). In this study, we apply a large battery of these fusion-partner probes to a cohort of confirmed *TFE3*-rearranged Xp11 translocation cancers by break-apart FISH, and correlate the subtype with clinicopathologic features. We also corroborate the results in 5 cases using RNA-sequencing.

MATERIALS AND METHODS

Case Selection and FISH analysis

The cases studied included 60 cases collected from the consultation files of 3 of the authors (PA, VER, CRA) and additional cases collected from the institutional files of The Johns Hopkins Medical Institutions and Memorial Sloan Kettering Cancer Center. Twenty of these cases were previously reported but without data as to fusion partner subtype in a prior study demonstrating the efficacy of *TFE3* break-apart FISH in renal tumor consultations (11). Two other cases of Xp11 translocation PEComa in this study were included from the original description of this entity (20) and one other case was reviewed by one author (PA) in consultation and subsequently reported by others (27). One case of melanotic Xp11 translocation renal cancer in this study was included in the original description of this entity (25) and two other cases were reviewed by one author (PA) in consultation and subsequently reported by others (28, 29). Immunohistochemistry for PAX8 (which is almost always negative in PEComa)(30) and cathepsin K (which is consistently positive in PEComa) was performed as previously described (12). For RCC, staging was performed using the American Joint Commission on Cancer Manual, 7th edition (31). This study was approved by the Institutional Review Boards at participating Institutions.

FISH on interphase nuclei from paraffin-embedded 4-micron sections was performed applying custom probes using bacterial artificial chromosomes (BAC), covering and flanking genes that were identified as potential fusion partners in the RNA-seq experiment. *TFE3* break-apart FISH was performed as previously described (11). BAC clones were chosen according to UCSC genome browser (http://genome.ucsc.edu), see Supplementary Table 1. The BAC clones were obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (CHORI)(Oakland, CA)(http://bacpac.chori.org). DNA from individual BACs was isolated according to the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. Slides were then incubated, washed, and mounted with DAPI in an antifade solution, as previously described (32). The genomic location of each BAC set was verified by hybridizing them to normal metaphase chromosomes. Two hundred successive nuclei were examined using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen,

Germany), controlled by Isis 5 software (Metasystems, Newton, MA). A positive score was interpreted when at least 20% of the nuclei showed a break-apart signal. Nuclei with incomplete set of signals were omitted from the score.

RNA-sequencing and Data Analysis

To verify FISH results in 5 selected cases with available material, RNA-seq was performed as described previously (5). Briefly, total RNA was extracted after xylene deparaffinization, using the AllPrep DNA/RNA FFPE Kit (Qiagen, Hilden, Germany). The quantity and integrity of the RNA was measured using the Agilent 2100 Bioanalyzer. One hundred nanograms of the RNA was applied for sequencing library preparation using the TruSeq RNA Access Library Prep Kit (Illumina, San Diego, USA) as per the manufacturer's protocol. Paired-end sequencing (75 bp×2) was performed using the MiSeq Reagent V3 Kit (150 cycles) and the MiSeq sequencing system (Illumina). STAR algorithm was employed for detection of any potential *TFE3* fusion. Bowtie2 was employed for alignment and mapping of short sequence reads to the human genome reference hg19 and the fusion transcript *SFPQ/PSF-TFE3.* Integrative Genomics Viewer (IGV) was employed for data visualization.

RESULTS

A total of 60 cases with known *TFE3* gene rearrangements demonstrated by *TFE3* break-apart FISH were analyzed for fusion partners. These included 47 Xp11 translocation RCC, 8 Xp11 translocation PEComa (2 renal, 6 non-renal), and 5 melanotic Xp11 translocation renal cancers. A fusion partner was identified in 53 cases (88%), including 18 *SFPQ (PSF),* 16 *PRCC,* 12 *ASPSCR1 (ASPL),* 6 *NONO,* and 1 *DVL2* (Figure 1). In the remaining 7 (12%) cases no gene partner was identified using the available probes, including custom BACs for the above genes and for additional less common *TFE3* gene partners reported, such as *CLTC, YAP1* (recently shown to be fused to *TFE3* in a subset of epithelioid hemangioendotheliomas) (33), or *PARP14*. The results for specific subtypes are presented according to the specific gene rearrangement identified.

NONO-TFE3 RCC (5 cases)

These five cases affected three males and two females (mean age 38.4 years, median 36 years) (Table 1, cases 1–5). Two cases presented as small localized RCCs, one each presented with regional lymph node and bone metastases, while another presented as localized stage one disease but developed lung metastases after two years. Morphologically, the tumors showed a combination of nested to papillary architecture, and predominantly clear cytoplasm. Four of five cases demonstrated psammomatous calcifications. Four of the five cases demonstrated nuclear palisading with sub-nuclear vacuoles, a pattern which mimics clear cell papillary RCC (34,35) (Figures 2,3). This pattern was focal (present in <50% of the tumor) in two cases and diffuse (present in >50% of the tumor) in the other two. In the fifth case, nuclear palisading resembled a trabecular architecture, leading to an initial impression of a neuroendocrine neoplasm. By immunohistochemistry, all five cases were immunoreactive for PAX8, but none expressed cathepsin K (PAX8+, cathepsin K−).

All 5 cases were negative for Carbonic Anhydrase IX (CA-IX). Two of five cases had rare cytokeratin 7 positive cells with the other three cases being completely negative.

NONO-TFE3 PEComa with Melanin Pigment (1 case)

This neoplasm presented as an orbital mass in a 20 year-old male (Table 1, case 6). The morphology was that of a nested epithelioid neoplasm, with clear to finely granular eosinophilic cytoplasm, typical of an Xp11 translocation PEComa, with the exception of abundant melanin pigment (Figure 4). By immunohistochemistry, this neoplasm had the opposite immunoreactivity of the *NONO-TFE3* RCC; being positive for cathepsin K, but not for PAX8 (PAX8−, cathepsin K+). Clinical follow-up on this case is not available.

DVL2-TFE3 RCC (1 case)

This neoplasm was a 14.5 cm renal tumor in a 73 year-old male. The tumor demonstrated papillary and solid architecture with focal sarcomatoid areas, had variably eosinophilic cytoplasm, and presented with right perirenal lymph node metastases (pT3N1). By immunohistochemistry, the neoplasm was positive for PAX8, but negative for cathepsin K and melan A (Figure 5). For comparison, we performed PAX8 and cathepsin K immunohistochemistry on a previously reported *DVL2-TFE3* PEComa, a 5.5 cm tumor located on the calf (22). The PEComa demonstrated the opposite pattern as the RCC with the same *DVL2-TFE3* gene fusion; it was PAX8 negative and cathepsin K positive (not shown).

SFPQ-TFE3 RCCs (7 cases)

Seven RCC harbored a *SFPQ-TFE3* gene fusion. These cases occurred in patients ranging from 19 – 63 years of age (mean 39, median 36), and 6 of 7 occurred in females (Table 2, cases 1–7). All cases were initially confined to the kidney. Case 1 presented as pT2 disease, had sarcomatoid morphology and later recurred in the retroperitoneum. Morphologically, the tumors showed a combination of nested to papillary architecture, and predominantly clear cytoplasm. All seven cases demonstrated psammomatous calcifications. Five of these seven cases demonstrated striking sub-nuclear vacuoles, similar to those seen in clear cell papillary RCC and (as shown above) in the RCC with *NONO-TFE3* gene fusion. In all five cases, this pattern was diffuse (present in >50% of the tumor). In areas in which the sub-nuclear vacuoles were less evident, the morphology was typically that of a nested epithelioid clear cell neoplasm with thin capillary vasculature, closely mimicking clear cell RCC (Figures 6, 7). Six of these 7 RCC were reactive for PAX8 but not for cathepsin K (PAX8+ cathepsin k −). The other case (case 6) was positive for PAX8 and focally positive for cathepsin K in cystic areas of the tumor. All 3 tested cases were negative for cytokeratin 7. Three of 4 cases were completely negative for CA-IX while one case demonstrated rare positive cells.

SFPQ-TFE3 PEComas (7 cases)

These neoplasms comprised 7 of the 8 Xp11 translocation PEComas available for study (the other case demonstrated a *NONO-TFE3* gene fusion described above). Six patients were females and 1 was male. Patient ages ranged from 4 – 46 years (mean 21.6, median 21). Sites of origin included the kidney (2 cases), renal sinus, uterus, bladder, thigh, and pelvis

(Table 2, cases 8–14). All cases demonstrated the morphology previously described in Xp11 translocation PEComa; specifically, a solid nested architecture featuring epithelioid cells with predominantly clear to focally eosinophilic cytoplasm. All cases analyzed were diffusely immunoreactive for cathepsin K, but negative for PAX8 (PAX8−, cathepsin k+) (Figure 8). All cases were immunoreactive for HMB45, but only four of six were immunoreactive for melan A. None of these cases demonstrated melanin pigment.

Both cases with adequate RNA for RNA seq demonstrated an *SFPQ-TFE3* gene fusion, fusing exon 9 of *SFPQ* with exon 6 of *TFE3* (Table 2, cases 12 and 14).

SFPQ-TFE3 Melanotic Xp11 translocation renal cancers (4 cases)

These comprised four of the five tested cases (the other case did not have an identifiable fusion partner). The patients ranged in age from 11 – 34 years (mean 27, median 24) (Table 2, cases 15–18). Two patients presented with disseminated metastatic disease. All of these cases demonstrated a solid nested architecture featuring epithelioid cells with predominantly clear to focally eosinophilic cytoplasm, along with melanin pigment readily identifiable on H&E sections. All of these neoplasms labeled diffusely for cathepsin K, and all were negative for PAX8 (PAX8− cathepsin k+). All 3 cases with adequate RNA for RNA seq demonstrated an *SFPQ-TFE3* gene fusion, fusing exon 9 of *SFPQ* with exon 6 of *TFE3* (Table 2, cases 16–18).

ASPSCR1-TFE3 RCC (12 cases)

These patients ranged in age from 15–73 years (mean 33.75, median 27), and nine were females and three males (Table 3). Morphologically, all neoplasms demonstrated the typical morphology of this subtype; specifically, nested to papillary architecture, voluminous clear to eosinophilic cytoplasm, and abundant psammoma bodies. Two of three cases with lymph nodes examined harbored lymph node metastases at diagnosis, and all nine cases tested were negative for cathepsin K.

PRCC-TFE3 RCC (16 cases)

These patients ranged in age from 5 – 63 years (mean 40.3, median 42), with an equal gender distribution, 8 females, 7 males, and one of unknown gender (Table 4). Morphologically, all tumors demonstrated the typical morphology of this subtype; specifically, they demonstrated compact nested to papillary architecture, clear to eosinophilic cytoplasm, and psammoma bodies. One case demonstrated palisading of nuclei and another demonstrated sub-nuclear vacuoles similar to RCC harboring *SFPQ-TFE3* and *NONO-TFE3* fusions, as described above. Seven of the eleven tested cases were immunoreactive for cathepsin K. Of note, two patients presented with hematogenous metastasis, which has not previously been reported in this subtype of Xp11 translocation RCC.

TFE3-Rearranged Neoplasms with Unknown Fusion Partner (7 cases)

The clinicopathologic features of these cases are summarized in Table 5. These included 6 Xp11 translocation RCC and 1 Melanotic Xp11 translocation cancer. Of note, all of the RCC were cathepsin K positive. Two of the RCC were extensively cystic and 2 had biphasic

morphology mimicking t(6;11) RCC as previously described (11). Adequate material for RNA seq was available from the Melanotic Xp11 translocation cancer; however, a *TFE3* fusion partner could not be identified

DISCUSSION

In this study, we apply combined molecular methodologies, FISH and RNA seq, to establish a detailed characterization of *TFE3* fusion partners in a large cohort of Xp11 translocation positive neoplasms. First, we provide the first morphologic description of the *NONO-TFE3* RCC. Previously, a single case of this entity had been reported (3), but a morphologic description and images were not provided. We found that this neoplasm and the *SFPQ-TFE3* RCC (see below) frequently demonstrate sub-nuclear vacuoles, leading to palisading of nuclei, similar to the appearance of clear cell papillary RCC. All five cases were immunoreactive for PAX8, but negative for cathepsin K. This immunoprofile contrasts with that of the Xp11 translocation PEComa harboring identical *NONO-TFE3* gene fusion (a fusion not previously reported in Xp11 translocation PEComa until now), which was conversely immunoreactive for cathepsin K but not for PAX8. We note that the presence of *NONO-TFE3* gene fusion can be suspected based on the pattern of *TFE3* rearrangement by FISH, showing constant, small gaps between the telomeric and centromeric TFE3 signals, in keeping with an inversion /intra-chromosomal fusion.

We also report the first RCC with a *DVL2-TFE3* gene fusion. This tumor had a variety of morphologic patterns, including oncocytic, tubular and sarcomatoid features. *DVL2-TFE3* fusion has been previously reported in an Xp11 translocation PEComa (22). In our study, similar to the *NONO-TFE3* neoplasms, we found that the *DVL2-TFE3* RCC was PAX8 positive and cathepsin K negative, whereas the *DVL2-TFE3* PEComa was cathepsin K positive and PAX8 negative.

We identified 18 neoplasms in this study harboring *SFPQ-TFE3* gene fusions. Prior studies have suggested significant overlap among Xp11 translocation RCC, Xp11 translocation PEComa, and melanotic Xp11 translocation renal cancers bearing the *SFPQ-TFE3* gene fusion. Some have grouped all neoplasms with the *SFPQ-TFE3* gene fusion together (36), and suggested that the distinction may be arbitrary. We have seen cases in which the same renal tumor has been seen by experts at several different major academic centers, and been classified as RCC by some and PEComa by others. Along these lines, we originally classified one of the cases in this study as an RCC based on its nested epithelioid morphology and renal location, but on re-review of the morphology and immunohistochemical profile reclassified it as a PEComa (Table 2, case 8). A complete immunohistochemical profile of tumors classified as RCC with the *SFPQ-TFE3* gene fusion has not been reported. We show herein that RCC with the *SFPQ-TFE3* gene fusion are immunoreactive for PAX8 and almost always negative for cathepsin K, which distinguishes them from Xp11 translocation PEComa and melanotic Xp11 translocation renal cancer, and frequently have a distinctive morphology featuring sub-nuclear vacuoles and nuclear palisading, similar to that seen in the *NONO-TFE3* RCC. The similar morphology of the *SPFQ-TFE3* and *NONO-TFE3* RCC is intriguing given the highly overlapping functions of *SFPQ* and *NONO* in RNA processing, and the fact that they together form protein

complexes (37). We note that, in retrospect, subnuclear vacuoles are readily seen in the published images provided in 4 case reports of *SFPQ-TFE3* RCC, though not commented on in these publications (38–41). The frequent sub-nuclear vacuolization seen indicates that these Xp11 translocation RCC should also be considered in the differential diagnosis of clear cell papillary RCC. Unlike clear cell papillary RCC, *SFPQ-TFE3* and *NONO-TFE3* RCC frequently occur in younger patients, frequently have psammomatous calcifications, and show no or minimal immunoreactivity for cytokeratin 7 and CA-IX. We also found that the *SFPQ-TFE3* RCC frequently mimics clear cell RCC to a striking degree. In fact, two of the cases in our study were previously diagnosed as clear cell RCC by experienced surgical pathologists with extensive expertise in urologic pathology consultations before they were reviewed in the course of this study. This morphologic mimicry of clear cell RCC also explains the observation that all 5 Xp11 translocation RCC mistakenly included in the cancer genome atlas (TCGA) sequencing study of clear cell RCC contained the *SFPQ-TFE3* RCC fusion (42). Based on these findings, we believe that the *SFPQ-TFE3* RCC are the Xp11 translocation RCC most likely to mimic clear cell RCC. The frequently younger patient age, focal papillary architecture, psammomatous calcification, and absence of or minimal labeling for CA-IX are clues to the diagnosis of *SFPQ-TFE3* RCC.

The usual PAX8-positive cathepsin K-negative immunoprofile of the *SFPQ-TFE3* RCC contrasts with those of the Xp11 translocation PEComa and melanotic Xp11 translocation RCC that harbor the same gene fusion, both of which were consistently cathepsin K positive and PAX8 negative. These findings suggest that the *SFPQ-TFE3* RCC are a distinctive entity which can be separated in the majority of cases from Xp11 translocation PEComa and melanotic Xp11 translocation cancers. The latter two neoplasms share a similar immunoprofile (PAX8 negative, cathepsin K positive), which supports our view and that of others that melanotic Xp11 translocation renal cancers likely represent a variant of or part of the spectrum of Xp11 translocation PEComa. Further evidence of overlap is the presence of melanin in several extrarenal Xp11 translocation neoplasms, including the *NONO-TFE3* PEComa in this study, an *SFPQ-TFE3* PEComa of the pancreas, pelvis and cervix reported by Rao et al (24), along with an ovarian melanotic Xp11 translocation neoplasm previously reported in the literature (43). While the phenotype of the Xp11 translocation RCC and Xp11 translocation PEComas is distinct at the immunohistochemical level, it is clear that these lesions are genetically related and can be considered part of a family of cancers driven by *TFE3* gene fusions. This suggests the potential for utilization of novel targeted therapies that are effective against one member against the others.

Our data on RCC with the *ASPCR1-TFE3* and *PRCC-TFE3* RCC adds supporting data to the published findings. We had previously shown that cathepsin K is frequently positive in the *PRCC-TFE3* RCC but consistently negative in the *ASPCR1-TFE3* RCC, a finding which is confirmed in this study (14). Two of three *ASPSCR1-TFE3* RCC in which lymph nodes were resected were associated with lymph node metastasis, which corroborates tendency to lymph node involvement we previously described in this subtype (16). Furthermore, 2 patients with small, seemingly localized *ASPCR1-TFE3* RCC who did not initially undergo node sampling at presentation (pT1NX) later recurred with involvement of retroperitoneal lymph nodes, suggesting the possibility that nodal sampling at diagnosis could have impacted outcome. A previous review of the literature showed that *PRCC-TFE3* RCC

typically present at lower stage than do the *ASPCR1-TFE3* RCC (16), but in this study we report the first two cases of *PRCC-TFE3* RCC which presented with metastatic disease. We note that neither of the *ASPCR1-TFE3* and *PRCC-TFE3* gene fusions was or has previously been identified in an Xp11 translocation PEComa. The *ASPCR1-TFE3* gene fusion is of course characteristic of the mesenchymal neoplasm alveolar soft part sarcoma (44), but there is no currently identified mesenchymal counterpart to the *PRCC-TFE3* RCC. Of note, the *PRCC-TFE3* RCC is the one known subtype of Xp11 translocation RCC which does express cathepsin K frequently, whereas our study shows that other Xp11 translocation RCC are typically cathepsin k negative and have a mesenchymal counterpart with the identical gene fusion that is cathepsin k positive (Table 6). It should be noted, however, that all of the Xp11 RCC in this study which do not as of now demonstrate a known fusion partner were positive for cathepsin K, suggesting that the as yet unknown *TFE3* fusion partners involved in these cases may function similarly to *PRCC*.

Given the variable clinical presentation and morphologic appearances described in this and other manuscripts, one could be tempted to conclude that all RCCs be worked up by molecular techniques to exclude Xp11 translocation RCC. Since the prognosis for Xp11 translocation RCC is similar to that of clear cell RCC, a missed diagnosis of a localized Xp11 translocation RCC as clear cell papillary RCC would result in the patient receiving an inappropriately optimistic report of their prognosis. For cases which present with metastatic disease, the diagnosis of Xp11 translocation RCC would make a patient ineligible for some treatments designed to specifically target clear cell RCC and eligible for treatments that target cancers with *TFE3* gene fusions. However, we believe that comprehensive molecular analysis of all RCCs is not cost effective. Our view is that RCCs with classic clinical presentation and morphology that is typical of the specific common subtypes of RCC (such as clear RCC, papillary RCC, and chromophobe RCC) do not require immunohistochemical stains or molecular analysis for diagnosis: the diagnosis can be comfortably reached on routine H&E sections. For other cases in which the morphology or clinical presentation is slightly unusual, we typically perform a limited immunohistochemical profile (such as cytokeratin 7, CA-IX and cathepsin K) to support or refute the suspected diagnosis. If this does not clarify the diagnosis, further workup is indicated. We do not routinely perform *TFE3* FISH unless there is a strong clinical suspicion (age less than 30 years), highly suggestive morphology (such as papillary architecture with clear cells and psammoma bodies), or highly suggestive immunohistochemical results (such as diffuse cathepsin K immunoreactivity). We also perform *TFE3* FISH for many unclassified RCCs, given the morphologic variability demonstrated by Xp11 translocation RCC including its ability to have a non-descript high grade RCC appearance.

In summary, our study highlights the ability of subset of Xp11 translocation RCC to mimic clear cell papillary RCC and clear cell RCC. Clinical clues such as young age, morphologic clues such as the presence of psammoma bodies, and immunohistochemical clues such as minimal immunoreactivity for CA-IX should suggest the possibility of Xp11 translocation RCC. Our study also highlights the different immunohistochemical phenotypes of Xp11 translocation RCC and PEComas that harbor the same gene fusion. Despite morphologic overlap, PAX8 and cathepsin K can distinguish most cases. We also highlight the strong

association of cathepsin K immunoreactivity in Xp11 translocation RCC with the presence of a *PRCC-TFE3* gene fusion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Norman Barker MA, MS, RBP for expert photographic assistance. 1-18-16

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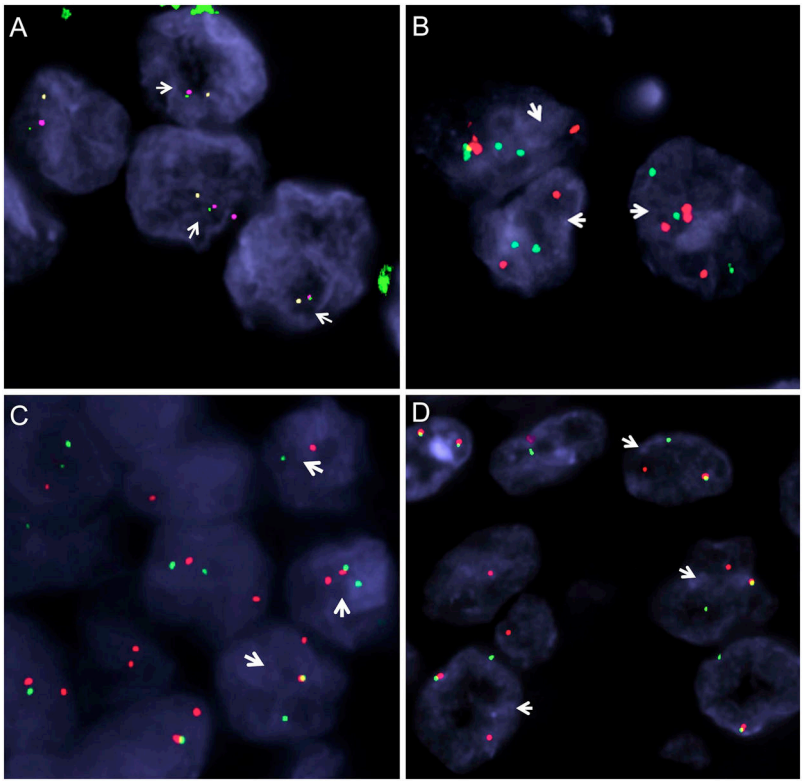

Figure 1.

FISH analysis of TFE3 fusion partner genes. All four neoplasms (A–D) in this composite image demonstrated TFE3 gene rearrangements by FISH. A. RCC with *NONO-TFE3* fusion (Table 1, case 3): 3-color FISH fusion assay shows the 5′ *NONO* (red, centromeric) signal is fused to the 3′*TFE3* probes (green, telomeric); while the 5′TFE3 probe (orange, centromeric) is split apart. B. *DVL2-TFE3* fusion positive RCC: arrows show 3 cells with *DVL2* break-apart signals (red, centromeric; green, telomeric); C. RCC with *SFPQ-TFE3* fusion (Table 4, case 4): arrows show 3 cells demonstrating split *SFPQ* signals (red, centromeric; green, telomeric). D. RCC with *PRCC-TFE3* fusion (Table 4, case 4): arrows show 3 cells with break-apart *PRCC* signals (red, centromeric; green, telomeric).

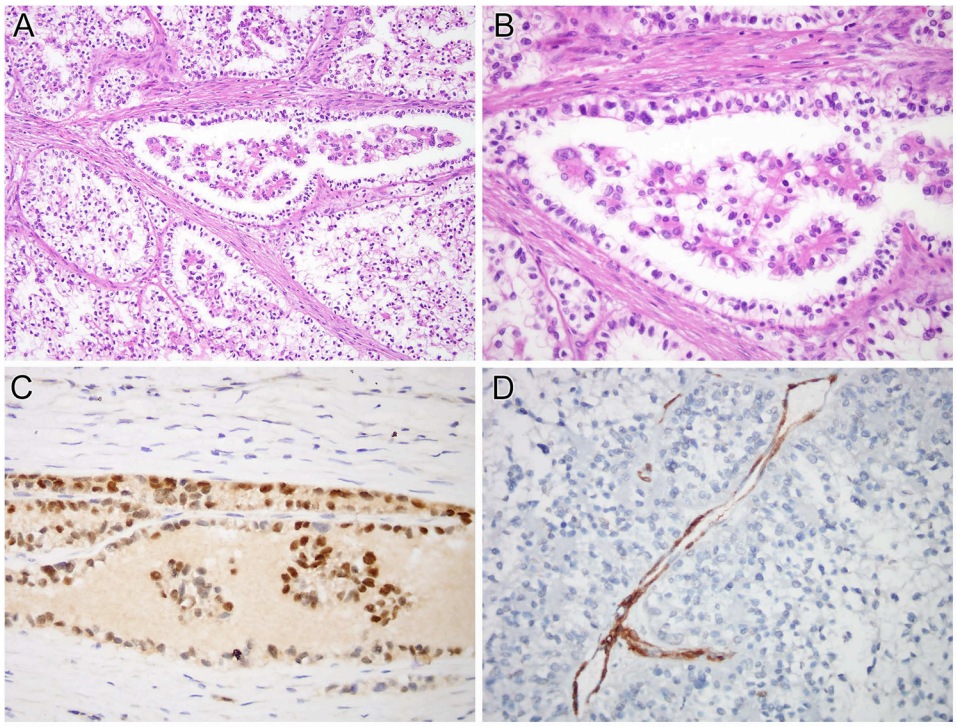

Figure 2.

NONO-TFE3 RCC (Table 1, case 1). A and B, this neoplasm demonstrated nested to papillary architecture. The neoplastic cells have predominantly clear cytoplasm and demonstrate sub-nuclear vacuolization, leading to apical palisading of nuclei. The neoplastic cells demonstrate nuclear labeling for PAX8 (C) but not for cathepsin K (D). Note the intact labeling of endothelial cells as an internal control for cathepsin K labeling.

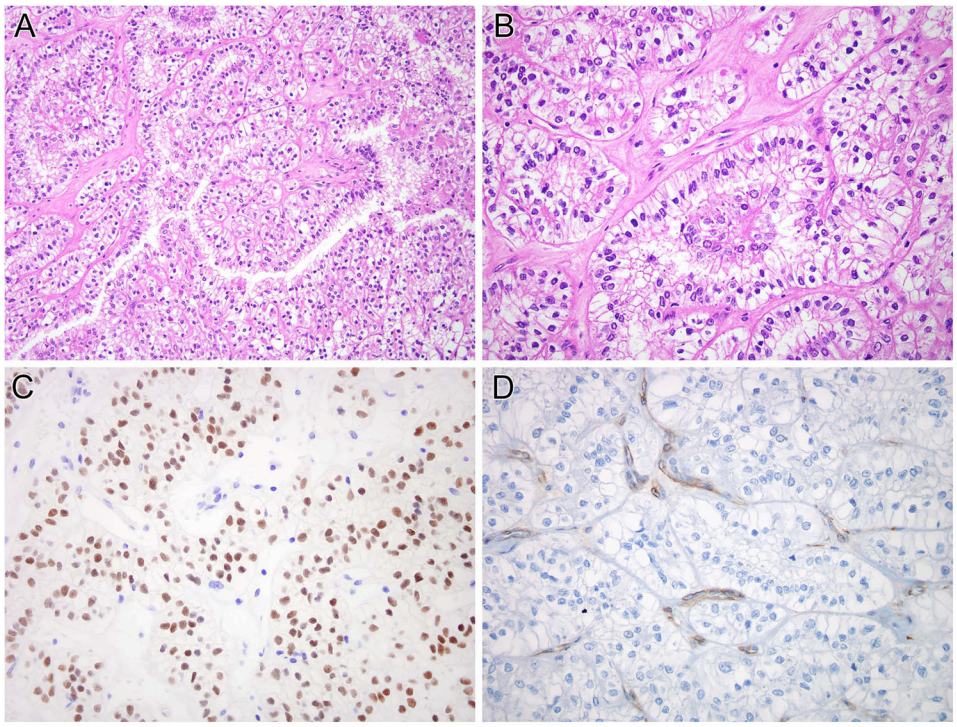

Figure 3.

NONO-TFE3 RCC (Table 1, case 4). A and B, this neoplasm demonstrated nested to papillary architecture. The neoplastic cells have predominantly clear cytoplasm and demonstrate sub-nuclear vacuolization, leading to apical palisading of nuclei. The neoplastic cells demonstrate nuclear labeling for PAX8 (C) but not for cathepsin K (D). Note the intact labeling of endothelial cells as an internal control for cathepsin K labeling.

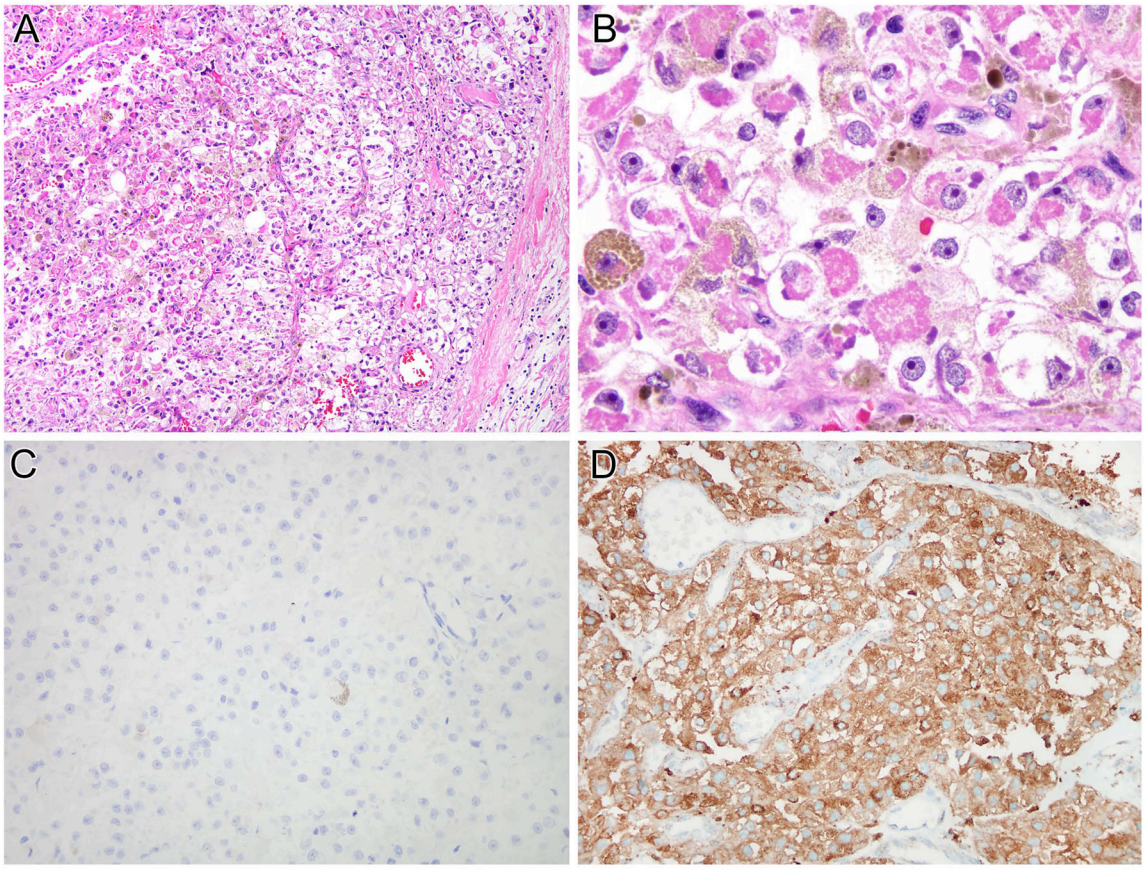

Figure 4.

NONO-TFE3 melanotic PEComa (Table 1, case 6). A and B, this is a neoplasm with nested to alveolar architecture, which features epithelioid cells with clear to finely granular eosinophilic cytoplasm. Fine pigment which proves to be melanin is present in the cytoplasm. The neoplasm was immunoreactive for melan A (not shown). The neoplasm does not label for PAX8 (C) but shows diffuse immunoreactivity for cathepsin K (D).

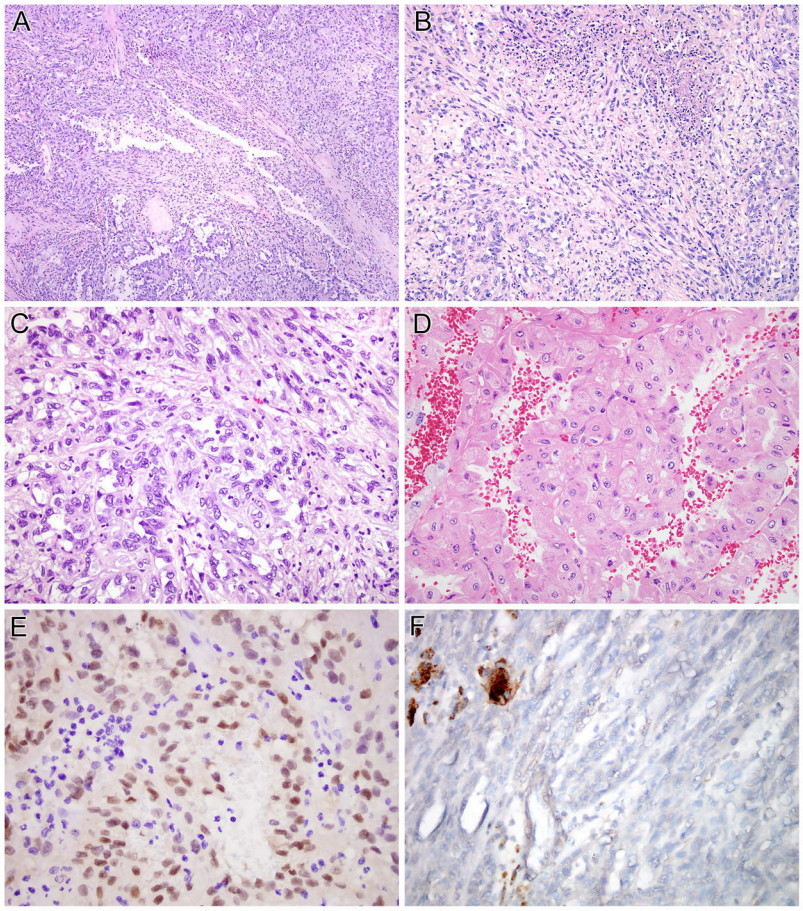

Figure 5.

DVL2-TFE3 RCC. This neoplasm demonstrates a variety of morphologic patterns. Much of the neoplasm has basophilic to pale cytoplasm, and demonstrates tubular and papillary architecture (A) each merges with sarcomatoid areas (B, C). Other areas on the same slides demonstrated more oncocytic cytoplasm (D). The neoplasm demonstrated nuclear immunoreactivity for PAX8 (E) but did not label for cathepsin K (F). Note the intact labeling of capillaries and associated macrophages as an internal control for cathepsin K labeling.

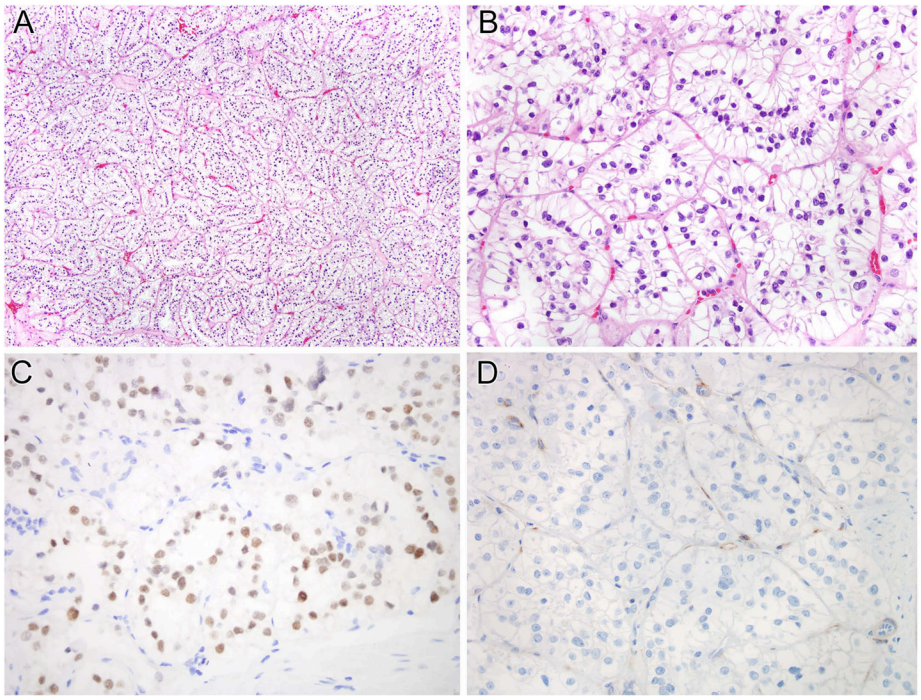

Figure 6.

SFPQ-TFE3 renal cell carcinoma (Table 2, case 5). This neoplasm demonstrated solid nested architecture (A and B), and demonstrated abundant clear cytoplasm with prominent sub-nuclear vacuoles leading to apical palisading of nuclei. This case was originally classified as a clear cell RCC. The neoplasm demonstrated nuclear labeling for PAX8 (C) but was negative for cathepsin K (D). Note the intact staining of endothelial cells as an internal control for cathepsin K labeling.

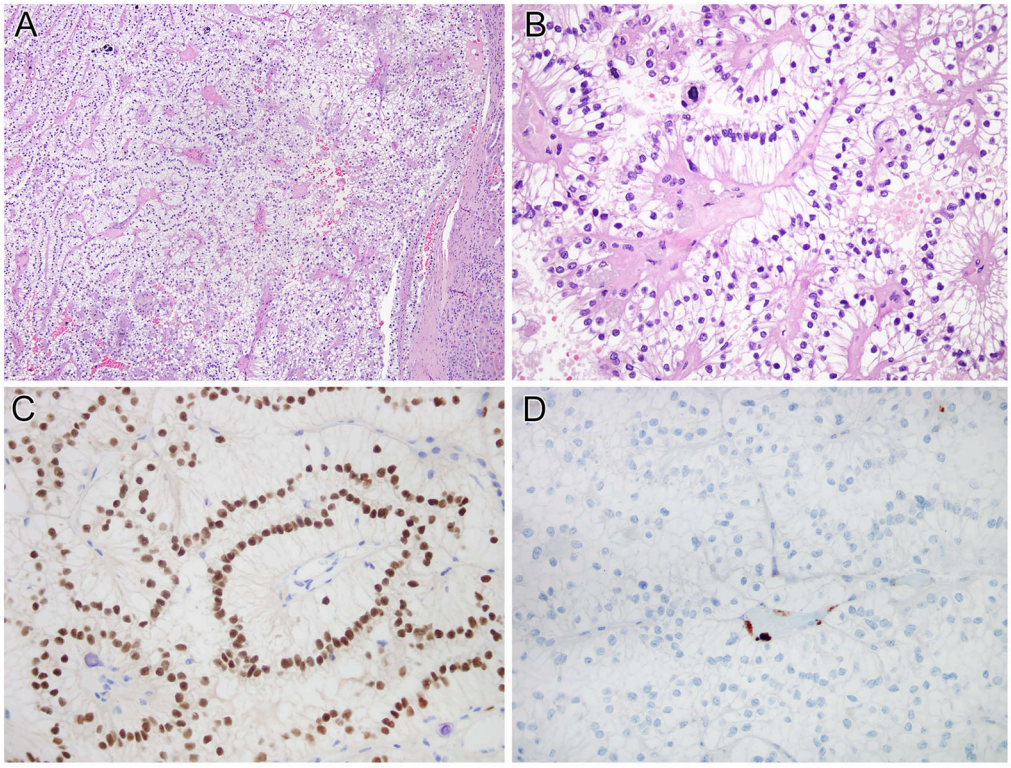

Figure 7.

SFPQ-TFE3 renal cell carcinoma (Table 2, case 4). A and B, this neoplasm demonstrated solid nested to papillary architecture, and striking sub-nuclear vacuoles leading to apical palisading of nuclei. The neoplasm was diffusely immunoreactive for PAX8 but not for cathepsin K. Note the intact staining of endothelial cells as an internal control for cathepsin K labeling.

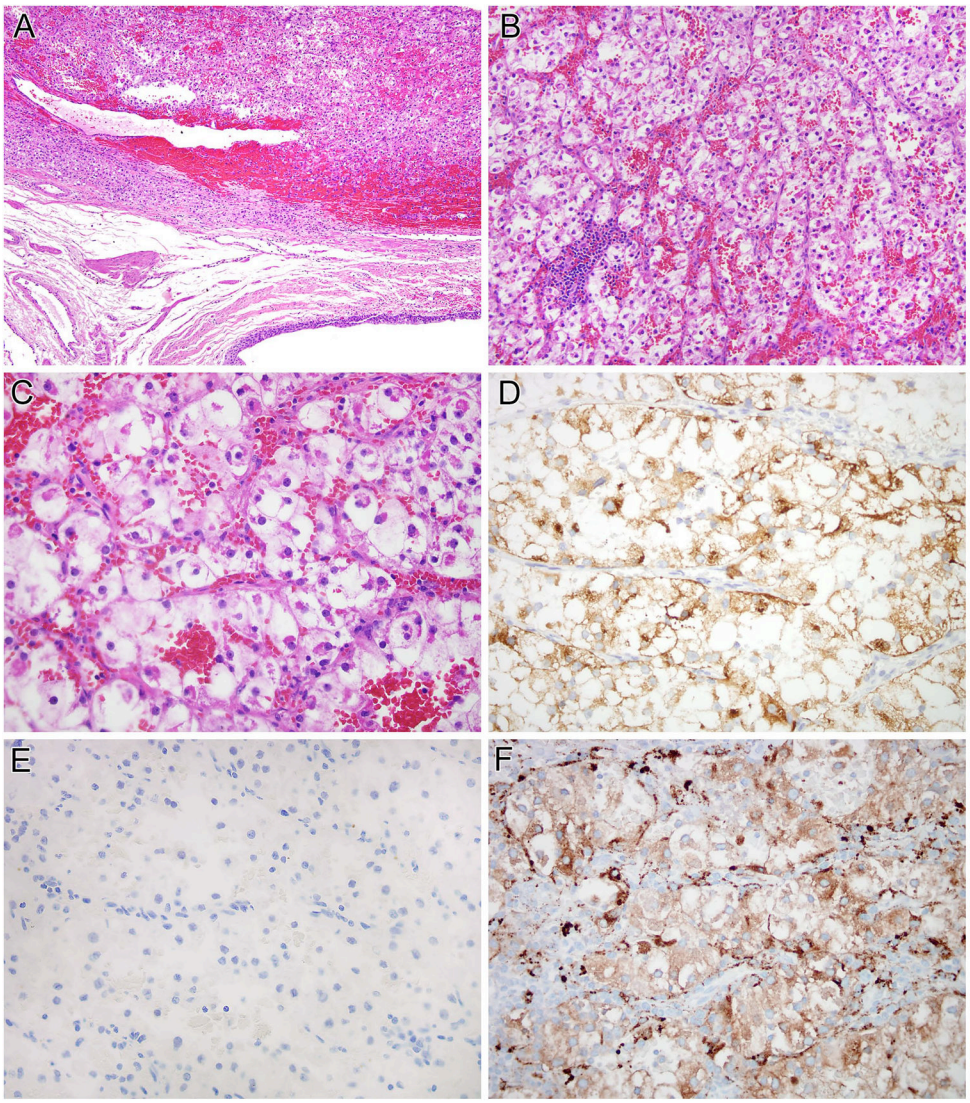

Figure 8.

SPFQ-TFE3 PEComa. (Table 2, case 9). This neoplasm was centered in the renal pelvis (A). The neoplasm was highly vascular, and had a nested to an alveolar architecture (B). The neoplastic cells had abundant clear to finely granular eosinophilic cytoplasm (C). The neoplasm was negative for cytokeratins, but showed labeling for HMB45 (D). The neoplasm was negative for PAX8 (E) but demonstrated diffuse labeling for cathepsin K (F).

Table 1

NONO-TFE3 Cases

Case	Age/Sex	Tumor Type	Size and Stage	Immunohistochemistry	Comment	Other publication
1	36/F	RCC	4.7cm, pT3NXM1 (bone), IV	PAX8+, HMB45 focal+; Cytokeratin 7, CA-IX, EMA, MiTF, Mart1, Cathepsin K−	Presented with back pain	Reference 11, Case 6
2	26/M	RCC	4cm, pT1NX, I	PAX8+; Cytokeratin focal+; Cytokeratin 7 rare cells +; Cathepsin K, Melan A, HMB45, CA-IX −	Lung metastasis after 2 years	Reference 11, Case 10
3	51/M	RCC	1.4cm, pT1NX, I (partial nephrectomy)	PAX8+; Racemase focal+; Cytokeratin 7, CA-IX, Cathepsin K, Vimentin, Cytokeratin, EMA−		Reference 11, Case 21
4	29/M	RCC	3.5cm, pT1NX, I	PAX8+; Cathepsin K, Cytokeratin 7, CA-IX−		Reference 11, Case 27
5	50/F	RCC	9cm, pT3N1, III	PAX8+; EMA+; HMB45 focal +; Cytokeratin 7 rare cells +; Cathepsin K, Vimentin, CA-IX−;		
6	20/M	Orbital Xp11 PEComa	Unknown	Cathepsin K+, HMB45+; PAX8, Desmin, Actin, Cytokeratin, Myogenin, S100−	Melanin Pigment	

Table 2

SFPQ-TFE3 Cases

Case	Age/Sex	Tumor Type	Size and Stage	Immunohistochemistry	Comment	Other publication
1	36/F	RCC	7.8cm, pT2NXMX, II	PAX8+; Cathepsin K−	Sarcomatoid, recurred in retroperitoneum after 3 years	Reference 11, Case 2
2	63/F	RCC	3.5cm, pT1NX, I (partial nephrectomy)	PAX8, Racemase +; Cathepsin K, Cytokeratin 7, CA-IX−		
3	32/F	RCC	4.7cm, pT1NX, I (partial nephrectomy)	PAX8, Racemase+; Cathepsin K, Cytokeratin 7, CA-IX, EMA−	Ipsilateral partial nephrectomy 4 years later for clear cell RCC	
4	24/F	RCC	3cm, pT1NX, I (partial nephrectomy)	PAX8, CD10, RCC marker, Racemase+; CA-IX rare cells +; Cathepsin K, CK7−,		
5	48/F	RCC	4cm, pT1NX, I	PAX8+; Cathepsin K, Vimentin, CA-IX−		
6	51/F	RCC	7.4cm, pT2NX, II (partial nephrectomy)	PAX8, CD10+; Cathepsin K, Cytokeratin AE1/3 focal+; HMB45, Melan A−		
7	19/M	RCC	1.6cm, pT1NX, I (partial nephrectomy)	PAX8+; Cathepsin K−		
8	35/F	Xp11 PEComa	Kidney, pT3aN1, clinical stage IV	Cathepsin K, HMB45+; PAX8−, Cytokeratin−	Multiple copies of fusion	Reference 11, Case 3
9	4/F	Xp11 PEComa	1.3cm, Renal sinus	Cathepsin K+, HMB45+; PAX8, Cytokeratin, S100 Melan A, EMA, RCC marker−		
10	27/F	Xp11 PEComa	Bladder	Cathepsin K, HMB45+; PAX8, Melan A, Desmin, Actin, S100−	Developed pelvic lymph node metastases at 6 months	Reference 27
11	9/M	Xp11 PEComa	7cm, Kidney, pT3N1 clinical stage IV	Cathepsin K, HMB45, Melan A+; PAX8, Cytokeratin, Desmin, S100 protein, CD34−		
12	46/F	Xp11 PEComa	6.5cm, Thigh	Cathepsin K+; PAX8−	*SFPQ exon 9-TFE3 exon 6 fusion*	Reference 20, Case 4.
13	21/F	Xp11 PEComa	6cm, Pelvis	Cathepsin K+; HMB45 focal+; PAX8, Melan A, Actin, Desmin, Cytokeratin−		
14	9/F	Xp11 PEComa	5cm, Uterus. Lymph node metastases at diagnosis.	Cathepsin K, HMB45+; PAX8, Melan A, S100, Desmin, Actin−	*SFPQ exon 9- TFE3 exon 6 fusion*	Reference 20, Case 2.
15	11/M	Melanotic Xp11 Renal Cancer	21cm, pT3N1M1, IV	Cathepsin K, Melan A, HMB45+; PAX8, Cytokeratin, RCC marker, Actin, Desmin, S100−		Reference 25, case 1
16	34/F	Melanotic Xp11 Renal Cancer	4.8cm, pT1NXMX, I (partial nephrectomy)	Cathepsin K, HMB45+; PAX8, CD10, Cytokeratin, EMA, Desmin,−	*SFPQ exon 9- TFE3 exon 6 fusion*	Reference 28.
17	30/F	Melanotic Xp11 Renal Cancer	12.5cm, pT3NX (clinically stage IV, thrombus in inferior vena cava and pulmonary artery)	Cathepsin K, HMB45, Melan A+; PAX8, Cytokeratin, CD10, Desmin, S100−	*SFPQ exon 9- TFE3 exon 6 fusion*	Reference 29

Case	Age/Sex	Tumor Type	Size and Stage	Immunohistochemistry	Comment	Other publication
18	34/M	Melanotic Xp11 Renal Cancer	9.7cm pT2NX, II	Cathepsin K, HMB45+; Melan A focal+; PAX8, Cytokeratin, S100−	*SFPQ exon 9- TFE3 exon 6 fusion*	

Table 3

ASPSCR1-TFE3 Renal Cell Carcinoma

Case	Age/Sex	Size and Stage	Immunohistochemistry	Comment	Other publication
1	20/F	12cm, pT2NXM1, IV	PAX8+; Cathepsin K−		Reference 11, Case 4
2	67/M	5.8cm, pT1bNX, I	PAX8+; Cathepsin K, HMB45−		Reference 11, Case 8
3	23/F	2cm, pT1aN1MX, III	Cytokeratin 7+, Vimentin focal+; Cathepsin K, CA-IX, HMB45−		Reference 11, Case 14
4	17/F	5cm, pT1bNXMX, I	CD10+, RCC+; Cathepsin K−		Reference 11, Case 29
5	39/F	14cm, pT3NXM1, IV	RCC marker, CD10+; Cathepsin K−	Diagnosed as urothelial carcinoma	Reference 11, Case 3
6	41/F	6.5cm, pT1 NXMX, I	PAX8+; Cathepsin K−	Metastasized to spine after 1 year	
7	28/F	8cm, pT2N0, II	PAX8+; Cathepsin K−	Hematuria and sickle cell trait, retroperitoneal recurrence 3 years later	
8	33/M	2.4cm, pT3aN1, III	CD10+	s/p chemotherapy for lymphoma, also has sarcoma of mediastinum	
9	26/M	7cm, pT3N1, III	PAX8+; Cathepsin K−		
10.	23/F	4cm, pT1NX, I (partial nephrectomy)		Recurred after 1 year→pT3N1 radical nephrectomy (matted lymph nodes)	
11	73/F	2.2cm, pT1NX, I	PAX8, AE1/3, CD10+; CA-IX, HMB45−	Retroperitoneal lymph node recurrence 7 years later	
12	15/F	pTXNXM1, IV	PAX8+; Cathepsin K, CA-IX−	Presented with cervical lymph node metastases	

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Table 4

PRCC-TFE3 Renal Cell Carcinoma

Case	Age/Sex	Size and Stage	Immunohistochemistry	Comment	Other publication
1.	19/M	12 cm pT3NXM1 (bone), IV	PAX8+, Cathepsin K+; Desmin, Vimentin focal+; HMB45−	Sarcomatoid	Reference 11, Case 7
2.	58/F	12cm, pT3NXMX, III	Cathepsin K+; CD10, Melan A focal+	Contralateral clear cell RCC	Reference 11, Case 13
3.	29/F	11.5cm, pT2NX, II	Racemase diffusely+; Cathepsin K, CA-IX, Vimentin focal +	Hematuria during pregnancy; nephrectomy during delivery	Reference 11, Case 20
4.	47/M	3.5cm, pT3NXMX, III (partial nephrectomy)	Cathepsin K−	Capsular invasion	Reference 11, Case 28
5.	21/F	9cm, pT2N1MX, III	Cathepsin K−	Cystic	Reference 11, Case 30
6.	30/M	3cm, pT1NXMX, I (partial nephrectomy)	Vimentin+; Cathepsin K−		
7.	Unknown	Unknown	Cathepsin K+		
8.	52/M	5cm, pT3N0M0 (involved inferior vena cava), III	Cathepsin K+	Developed bone metastasis 1.5 years later, died of disease	
9.	5/F	Renal mass with retroperitoneal lymphadenopathy			
10	48/F	3.7cm, pT1NX, I (partial nephrectomy)	Cathepsin K focal+		
11	35/F	Renal mass with retroperitoneal lymphadenopathy	Cathepsin K−	Core biopsy	
12	50/F	0.9cm, pT1NX, I (partial nephrectomy)	Cathepsin K, Racemase +; Cytokeratin 7 focal +, Melan A −	Incidental finding on imaging	
13	62/M	“Organ Confined” by report	PAX8+	Bladder recurrence at 12 years→Neck metastasis at 16 years	
14	37/M	8.5cm, pT3NX, III	RCC marker, CD10+; Cam5.2, AE1/3, Cytokeratin 903, HMB45, Melan A−		
15	63/F	5.3cm, pT1bNX, I	CD10, PAX8+, racemase+; CA-IX−		
16	49/M	11cm, pT3NXM1, IV (pelvis and intestinal wall)	CD10+; CK7, CK20−		

Table 5

TFE3-Rearranged Cases with Unknown Fusion Partner

Case	Age/Sex	Tumor Type	Size and Stage	Immunohistochemistry	Comment	Other publication
1	33/M	RCC	2.2cm, pT1NX, I (partial nephrectomy)	Cathepsin K, PAX8, Melan A+	Multilocular cystic	Reference 11, case 9
2	28/F	RCC	3.4cm pT1NXMX, I (partial nephrectomy)	Cathepsin K+		Reference 11, case 11
3	16/F	RCC	pT1aNXMX, I (partial nephrectomy)	PAX8, Cathepsin K, Cytokeratin, CD10+; HMB45, Melan A−	Highly cystic	Reference 11, Case 16
4	20/F	RCC	13.6cm, pT3N2M0, III	Cathepsin K, CD10+	Went on E2805 (sorafenib), developed pulmonary and paravertebral metastases at 2 years	Reference 11, case 26
5	46/F	RCC	3.7cm, pT3NX, III (partial nephrectomy)	Cathepsin K+; Cam5.2, Melan A, HMB45 focal+	Mimic t(6;11) RCC	
6	56/F	RCC	4.5cm, pT1NX, I (partial nephrectomy)	Cathepsin K, PAX8, Melan A, CD117+	Mimic t(6;11) RCC	
7	21/F	Melanotic Xp11 Renal cancer	pT3NX, III	Cathepsin K, HMB45, Melan A+; PAX8, S100, Vimentin, Actin−	Lung nodules 4 years later	

Table 6

Specific Recurrent *TFE3* Gene Fusions in Epithelial and Mesenchymal Neoplasms

Gene Fusion	Mesenchymal Neoplasm	Immunohistochemical Profile	Epithelial Neoplasm	Immunohistochemical Profile
ASPSCR1- TFE3	Alveolar Soft Part Sarcoma	PAX8-Cathepsin K+	*ASPSCR1- TFE3* RCC	PAX8+ Cathepsin K−
SFPQ-TFE3	Xp11 PEComa/ Melanotic Xp11 Cancer	PAX8-Cathepsin K+	*SFPQ-TFE3* RCC	PAX8+ Cathepsin K−*
NONO-TFE3	Xp11 PEComa/ Melanotic Xp11 Cancer	PAX8-Cathepsin K+	*NONO-TFE3* RCC	PAX8+ Cathepsin K−
DVL2-TFE3	Xp11 PEComa	PAX8-Cathepsin K+	*DVL2-TFE3* RCC	PAX8+ Cathepsin K−
PRCC-TFE3	ND	ND	*PRCC-TFE3* RCC	PAX8+ Cathepsin K +/−
YAP1-TFE3	Epithelioid Hemangioendothelioma Subset	Not studied	ND	ND

ND=not described; RCC=renal cell carcinoma

*One case was focally Cathepsin K positive