Int J Clin Exp Pathol 2013;6(11):2585-2590
www.ijcep.com /ISSN:1936-2625/IJCEP1309007

Case Report

A case of bilateral renal cell carcinoma associated with long-term dialysis showing false-positive immunoreactivity for TFE3 as Xp11 translocation renal cell carcinoma

Aiko Kurisaki-Arakawa[1], Tsuyoshi Saito[1], Michiko Takahashi[1], Keiko Mitani[1], Yuki Fukumura[1], Yoji Nagashima[2], Pedrum Argani[3], Takashi Yao[1]

[1]Department of Human Pathology, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo, Japan; [2]Department of Molecular Pathology, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama, Japan; [3]Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

Received September 3, 2013; Accepted September 18, 2013; Epub October 15, 2013; Published November 1, 2013

Abstract: Renal carcinomas associated with Xp11.2 translocations/transcription factor 3 (*TFE3*) gene fusion (Xp11 translocation RCC) are a rare subtype of renal cell carcinoma. A middle-aged Japanese man, who had a medical history of dialysis for more than 12 years, had bilateral renal cancers with a background of acquired cystic disease of the kidney and remarkable deposition of calcium oxalate in the tumorous area. The right renal tumor showed papillary architecture of clear cells with diffuse and strong immunoreactivity for TFE3 and focal and weak positivity for cathepsin K, suggesting a possibility of Xp11 translocation RCC. However, RT-PCR failed to detect any type of the reported fusion genes involving *TFE3*. Thus, the sample was sent for a *TFE3* break-apart FISH assay in a renal tumor consultation service, which reported no evidence of *TFE3* gene rearrangement. The right renal tumor was finally diagnosed as papillary renal cell carcinoma with cystic change. We report here a case of bilateral renal cell carcinoma in a patient undergoing long-term dialysis, which showed false-positive immunoreactivity for TFE3 immunostaining. Titration of TFE3 immunohistochemical staining (IHC) should be performed and cross-referenced with the FISH or RT-PCR results to avoid the misinterpretation of TFE3 IHC results.

Keywords: Bilateral renal cell carcinoma, long-term dialysis, TFE3 as Xp11 translocation, FISH, RT-PCR

Introduction

Renal carcinomas associated with Xp11.2 translocations/transcription factor 3 (*TFE3*) gene fusion (Xp11 translocation RCC) are a rare subtype of renal cell carcinomas [1-4]. This tumor is caused by several different translocations involving chromosome Xp11.2, resulting in various types of gene fusions involving *TFE3* [3-5].

This tumor occurs predominantly in children and young adults and sometimes in older adults [2-5]. Although the prognosis of this tumor is usually good for younger patients, it frequently metastasizes to lymph nodes, following a more aggressive clinical course in older patients [5]. Therefore, it is important, especially in cases involving elderly patients, to distinguish this tumor from other subtypes of renal cell carcinomas. However, the diagnosis is sometimes confusing as to whether this tumor is a true Xp11 translocation RCC or not, because the immunohistochemical staining (IHC) for TFE3 sometimes shows a false-positive result when an overly sensitive assay is performed or when the titration of TFE3 IHC is not enough. It can often result in enhanced detection of native TFE3 protein by IHC, because TFE3 is ubiquitously detected lightly in normal cells. We report a case of bilateral renal cell carcinoma in a patient undergoing long-term dialysis, which showed false-positive immunoreactivity for TFE3 IHC. We avoided the misdiagnosis of this

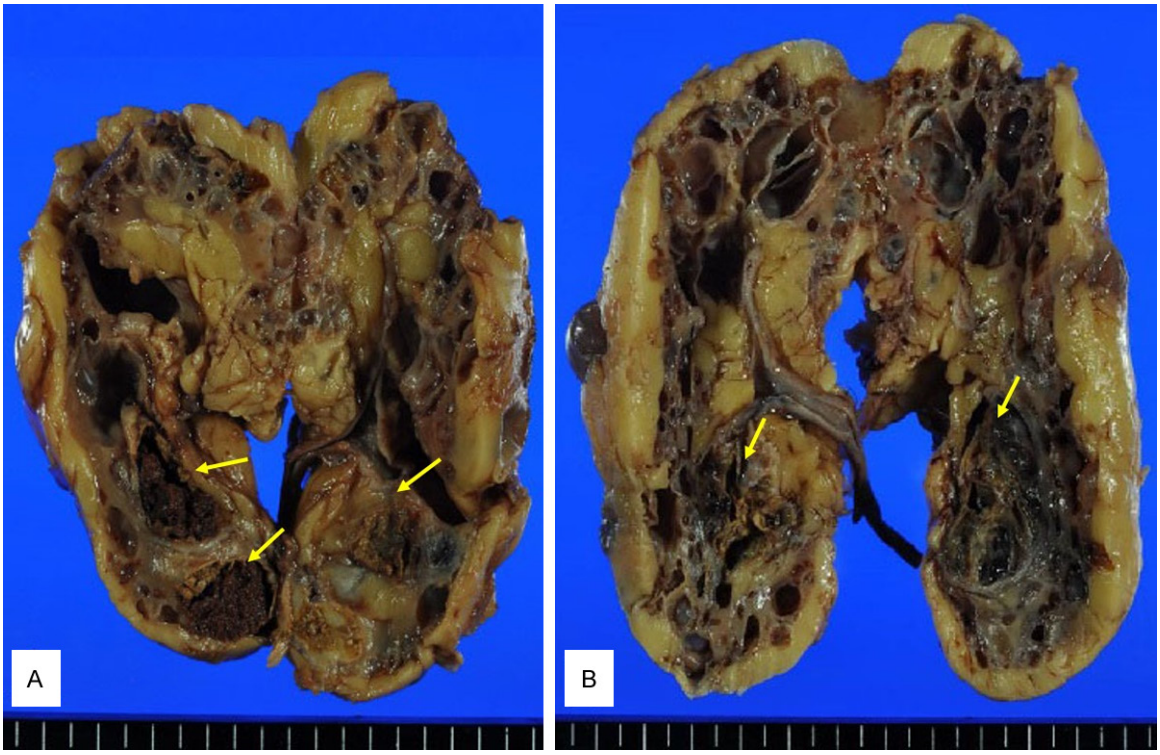

Figure 1. Grossly, right kidney tumor showed yellowish papillary lesion with hemorrhage in multiple cysts (A). Left renal tumor was located at the lower pole and is consisted of yellow-colored solid part in a multicystic lesion (B).

case by using a *TFE3* break-apart FISH assay in a renal tumor consultation service [6].

Case report

Clinical history

A 50-year-old Japanese man was admitted to our hospital because of right lateral abdominal pain. He had a medical history of dialysis for more than 12 years (5 years of hemodialysis, 7 years of peritoneal dialysis) owing to chronic renal failure from kidney disease of unknown origin. Abdominal computed tomography demonstrated a left renal tumor in the lower pole and a cystic tumor with a solid part in the lower pole of the right kidney. Distant metastasis was not suspected by general screening. Bilateral nephrectomy was performed under the clinical diagnosis of bilateral renal cancer.

Pathological findings

Grossly, the right kidney tumor showed a yellowish papillary lesion with hemorrhage in multiple cysts (**Figure 1A**). The left renal tumor was located at the lower pole and consisted of a yellow solid part in a multicystic lesion (**Figure 1B**).

Microscopically, left renal tumor cells had clear cytoplasm and round to oval nuclei. The cyst wall was lined by clear tumor cells (**Figure 2A**), and the solid tumor component was surrounded by those cystic parts (**Figure 2B**). Several calcium oxalate deposits were observed within the tumor. The tumor of the right kidney was composed of cystic architectures lined with tumor cells. The tumor cells were mixture of large eosinophilic cells and smaller columnar clear cells. The former possessed hyperchromatic large nuclei, whereas the later small pyknotic nuclei. Focally, the tumor formed a papillary architecture (**Figure 2C**), and oxalate crystals were scattered in the tumor (**Figure 2D**). Psammomatous calcifications were not observed throughout the tumor. Eosinophilic tumor cells had middle-sized and prominent nucleoli (Fuhrman grade 3). The background of the nontumorous area of both kidneys showed acquired cystic disease of the kidney (ACDK).

Immunohistochemical study

Accurate diagnosis of Xp11 translocation RCC requires detection of a diffuse and strong nuclear immunoreactivity for TFE3 [7]. Immu-

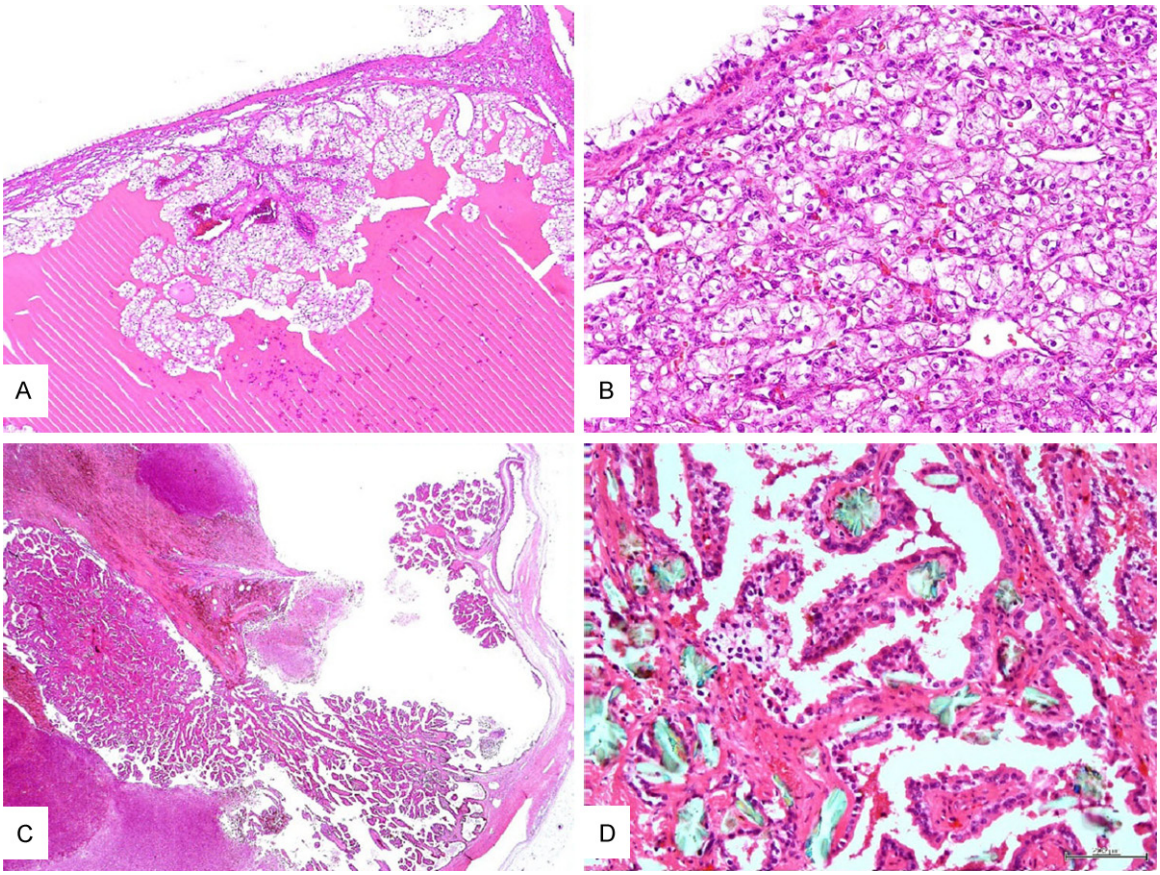

Figure 2. A and B: Hematoxylin and eosin staining showing left renal cyst walls lined by clear tumor cells, with focal papillary projection into the lumen (A) and the solid part of the tumor (B). C: Right renal tumor showing papillary formation within the cyst. D: Numerous calcium oxalate deposits in the fibrovascular cores of the tumor.

nohistochemical staining for TFE3 was performed on bilateral renal cancer. First, slides were deparaffinized and hydrated, and then sections were autoclaved in 10 mmol/L citrate buffer (pH 6.0). After protein blocking (15 min) and peroxide blocking (30 min), the slides were incubated overnight at 4°C using goat polyclonal antihuman TFE3 (sc-5958, dilution 1:200; Santa Cruz Biotechnology, Santa Cruz, CA), followed by secondary antibody incubation with biotinylated rabbit anti-goat antibody (1:500; Vector Laboratories, Burlingame, CA) for 10 min. The streptavidin-biotin-peroxidase complex technique and 3,3'-diaminobenzidine as the chromogen substrate were used for IHC. The slide was counterstained with hematoxylin, dehydrated, and cover-slipped.

An overly sensitive assay can result in enhanced detection of native TFE3 protein by IHC, as TFE3 is ubiquitously expressed in human tissue [7]. We used a case of alveolar soft part sarcoma as a positive control and a case of clear cell RCC as a negative control to prevent a false-positive reaction, as previously described [4].

Immunohistochemically, right renal tumor cells were diffusely and strongly positive for TFE3 (**Figure 3A**) and focally and weakly positive for cathepsin K (**Figure 3B**). The left renal tumor showed negative immunoreactivity for TFE3 and cathepsin K.

RNA extraction and RT-PCR

Based on the IHC findings, Xp11 translocation RCC was suspected and RT-PCR was performed to detect any of the reported fusion genes involving *TFE3*. RNA extraction from paraffin-embedded tissue was performed as previously described [8]. Primer sequences used in this study were also described previously [9]. After PCR, an aliquot of the PCR product was electrophoresed on a 2% agarose gel and stained with ethidium bromide. As a result, RT-PCR analysis

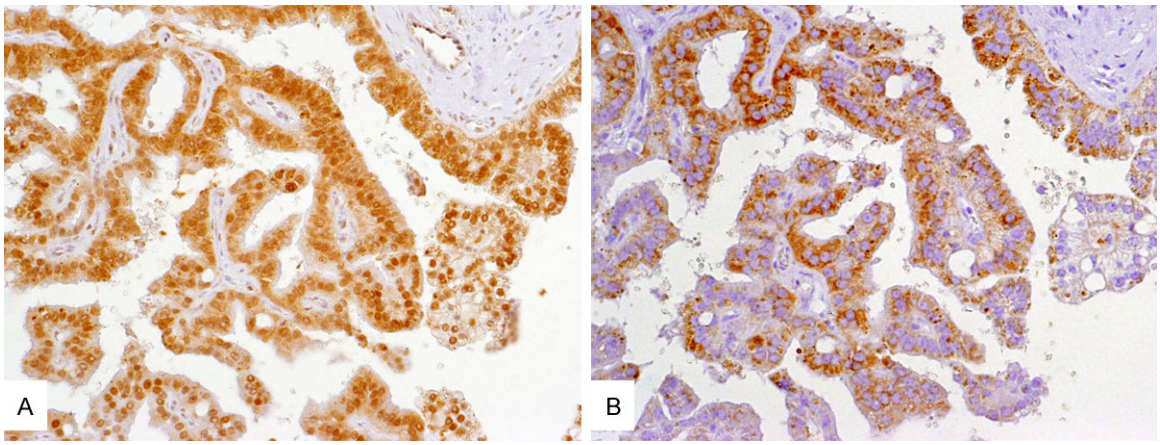

Figure 3. A and B: Immunohistochemical staining of TFE3 (A) and cathepsin K (B) on the right renal tumor. A: Diffuse strong nuclear immunoreactivity for TFE3. B: Focal weak cytoplasmic immunoreactivity for cathepsin K.

failed to detect any of the reported fusion genes involving *TFE3* in this tumor.

TFE3 FISH analysis

Because RT-PCR failed to detect any type of the reported fusion genes involving *TFE3*, despite the histological feature mimicking Xp11 translocation RCC together with IHC findings of diffuse strong immunoreactivity for TFE3 and focal weak positivity for cathepsin K, a *TFE3* break-apart FISH assay was performed. FISH analysis of TFE3 fusion status was done from paraffin-embedded tissues as described earlier [10]. Serial 5 μm unstained sections were cut from each paraffin block and subjected to a split FISH ("break-apart") assay with telomeric probes (indocarbocyanine, Cy3) and centromeric probes (fluorescein isothiocyanate, FITC) of TFE3 to determine if a TFE3 gene rearrangement was present. Here, the normal result is a combination (red and green) signal, whereas a TFE3 fusion results in a split signal. This assay has proven to be highly sensitive and specific for neoplasms harboring *TFE3* gene fusion. FISH showed no evidence of *TFE3* gene rearrangement for bilateral tumors. Although the classification of dialysis-associated RCC has not yet been fully established, the tumor should be considered as a mixed type of clear cell papillary and ACD-associated RCC [11].

Discussion

Xp11 translocation RCC in older adults is rare, but if it occurs, it frequently metastasizes to lymph nodes with a poor prognosis [5]. Therefore, it is important and necessary to diagnose correctly.

Only 1 case of Xp11 translocation RCC related to dialysis has been reported previously, although Xp11 translocation was not confirmed by either RT-PCR or FISH in the patient in this case [10], who had only a 5-year history of dialysis, and there was no intratumoral oxalate deposition. Conversely, the patient in our study had a medical history of dialysis for more than 12 years, and his bilateral kidneys showed ACKD with multifocal tumors and calcium oxalate deposition in both tumors. Retrospective review of our case raised a few histopathological points that are not in line with the Xp11 translocation RCC. First, the papillary architecture, which has been described as a distinctive feature for Xp11 translocation RCC, was quite focal, and second, psammomatous calcifications, reported to be observed in half of the cases, were not observed [5]. As previously described, conventional clear cell renal cell carcinoma is the most common histological type in patients undergoing dialysis for less than 10 years, whereas acquired cystic disease-associated renal cell carcinoma is predominant in those undergoing dialysis for more than 10 years [12]. In previous reports, renal cell carcinoma arising in patients with end-stage kidney disease often showed multifocal [11], cystic degeneration [13] and intratumoral oxalate deposition [11], in line with our case. The histology of our case was consistent with bilateral renal cell carcinoma arising in a patient with end-stage kidney disease with the background of ACDK.

Fusions involving *TFE3* constantly lead to overexpression of fusion protein, compared to native TFE3, and strong nuclear staining for TFE3 is shown to be a sensitive marker for Xp11 translocation RCC. However, a subset of the usual type of RCC may lead to the TFE3 activation through TFE3 amplification [14]. It has been demonstrated that TFE3 IHC often shows a false-positive result due to the detection of native TFE3 protein, as the TFE3 is ubiquitously distributed in the normal human tissue [6]. It is shown that immunohistochemical techniques such as excessive antigen retrieval, high antibody concentration, and excessive signal amplification could lead to the false-positive finding [7]. Furthermore, it has been demonstrated that all 13 tumors with moderate or strong TFE3 (n = 10) or cathepsin K (n = 7) immunoreactivity were FISH-positive [15]. In addition, it is noteworthy that none had positive IHC but negative FISH results [15]. Taking these findings into consideration, immunoreactivity for TFE3 and cathepsin in our case seemed to be a false-positive result, specifically detecting native TFE3 protein with TFE3 IHC. In our IHC, the concentration of TFE3 antibody seemed to be higher (1:200), and leading to the false positive result in this case, as it was originally described as 1:600 [7].

We report here a case of renal cell carcinoma associated with ACKD which is partially masquerading a Xp11 translocation RCC. This case really emphasizes the importance of titrating TFE3 IHC along with cross-referencing with the FISH or RT-PCR results to avoid the misinterpretation of TFE3 IHC results.

Acknowledgements

This work was supported in part by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Science, Sports, and Culture (23590434 to T.S.), Tokyo, Japan.

Address correspondence to: Dr. Tsuyoshi Saito, Department of Human Pathology, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo, Japan. Tel: +81-3-3813-3111; Fax: +81-3-3813-3428; E-mail: tysaitou@juntendo.ac.jp

References

[1] Kuroda N, Tanaka A, Ohe C, Nagashima Y. Recent advances of immunohistochemistry for diagnosis of renal tumors. Pathol Int 2013; 63: 381-390.

[2] Elbe JN, Sauter G, Epstein JI, Sesterhenn IA. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon, France: IARC Press; 2004. pp: 37-38.

[3] Camparo P, Vasiliu V, Molinie V, Couturier J, Dykema KJ, Petillo D, Furge KA, Comperat EM, Lae M, Bouvier R, Boccon-Gibod L, Denoux Y, Ferlicot S, Forest E, Fromont G, Hintzy MC, Laghouati M, Sibony M, Tucker ML, Weber N, Teh BT, Vieillefond A. Renal translocation carcinomas. Clinicopathologic, immunohistochemical, and gene expression profiling analysis of 31 cases with a review of the literature. Am J Surg Pathol 2008; 35: 656-670.

[4] Argani P, Antonescu CR, Couturier J, Fournet JC, Sciot R, Debiec-Rychter M, Hutchinson B, Reuter VE, Boccon-Gibod L, Timmons C, Hafez N, Ladanyi M. PRCC-TFE3 renal carcinomas: morphologic, immunohistochemical, ultrastructural, and molecular analysis of an entity associated with the t(X;1)(p11.2;q21). Am J Surg Pathol 2002; 26: 1553-1566.

[5] Argani P, Olgac S, Tickoo SK, Goldfischer M, Moch H, Chan DY, Eble JN, Bonsib SM, Jimeno M, Lloreta J, Billis A, Hicks J, De Marzo AM, Reuter VE, Ladanyi M. Xp11 translocation renal cell carcinoma in adults: expanded clinical, pathologic, and genetic spectrum. Am J Surg Pathol 2007; 31: 1149-1160.

[6] Green WM, Yonescu R, Morsberger L, Morris K, Netto GJ, Epstein JI, Illei PB, Allaf M, Ladanyi M, Griffin CA, Argani P. Utilization of a TFE3 break-apart FISH assay in a renal tumor consultation service. Am J Surg Pathol 2013; 37: 1150-1163.

[7] Argani P, Lal P, Hutchinson B, Lui MY, Reuter VE, Ladanyi M. Abberant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions: a sensitive and specific immunohistochemical assay. Am J Surg Pathol 2003; 27: 750-761.

[8] Tsuji S, Hisaoka M, Morimitsu Y, Hashimoto H, Shimajiri S, Komiya S, Ushijima M, Nakamura T. Detection of SYT-SSX fusion transcripts in synovial sarcoma by reverse transcription-polymerase chain reaction using archival paraffin-embedded tissues. Am J Pathol 1998; 153: 1807-1812.

[9] Chang IW, Huang HY, Sung MT. Melanotic Xp11 translocation renal cancer: a case with PSF-TFE3 gene fusion and up-regulation of melanogenic transcripts. Am J Surg Pathol 2009; 33: 1894-1901.

[10] Aulmann S, Longerich T, Schirmacher P, Mechtersheimer G, Penzel R. Detection of the ASP-CR-TFE3 gene fusion in paraffin-embedded al-

veolar soft part sarcomas. Histopathology 2007; 50: 881-886.
[11] Tickoo SK, dePeralta-Venturina MN, Harik LR, Worcester HD, Salama ME, Young AN, Moch H, Amin MB. Spectrum of epithelial neoplasms in end-stage renal disease: an experience from 66 tumor-bearing kidneys with emphasis on histologic patterns distinct from those in sporadic adult renal neoplasia. Am J Surg Pathol 2006; 30: 141-153.
[12] Nouh MA, Kuroda N, Yamashita M, Hayashida Y, Yano T, Minakuchi J, Taniguchi S, Nomura I, Inui M, Sugimoto M, Kakehi Y. Renal cell carcinoma in patients with end-stage renal disease: relationship between histological type and duration of dialysis. BJU Int 2009; 105: 620-627.
[13] Choyke PL. Acquired cystic kidney disease. Eur Radiol 2000; 10: 1716-1721.
[14] Macher-Goeppinger S, Roth W, Wagener N, Hohenfellner M, Penzel R, Haferkamp A, Schirmacher P, Aulmann S. Molecular heterogeneity of TFE3 activation in renal cell carcinomas. Mod Pathol 2012; 25: 308-315.
[15] Rao Q, Williamson SR, Zhang S, Eble JN, Grignon DJ, Wang M, Zhou XJ, Huang W, Tan PH, Maclennan GT, Cheng L. TFE3 break-apart FISH has a higher sensitivity for Xp11.2 translocation-associated renal cell carcinoma compared with TFE3 or cathepsin K immunohistochemical staining alone: expanding the morphologic spectrum. Am J Surg Pathol 2013; 37: 804-815.