

## Original Article

# Detection of the *ASPL-TFE3* and *PRCC-TFE3* gene fusion in paraffin-embedded Xp11 translocation renal cell carcinomas

Zi-Yu Wang<sup>1</sup>, Qiu-Yuan Xia<sup>2</sup>, Shan-Shan Shi<sup>2</sup>, Sheng-Bing Ye<sup>2</sup>, Rui Li<sup>2</sup>, Heng-Hui Ma<sup>2</sup>, Zhen-Feng Lu<sup>2</sup>, Xiao-Jun Zhou<sup>2</sup>, Qiu Rao<sup>1,2</sup>

<sup>1</sup>School of Basic Medical Sciences, Nanjing University of Traditional Chinese Medicine, Nanjing, China; <sup>2</sup>Department of Pathology, Nanjing Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China

Received April 6, 2016; Accepted June 13, 2016; Epub November 1, 2016; Published November 15, 2016

**Abstract:** Xp11 translocation renal cell carcinomas (RCCs) are uncommon renal tumors, characterized by several different translocations involving the *TFE3* gene. In these diseases, the *TFE3* gene is fused by translocation to 1 of several other genes, including *ASPL*, *PRCC*, *NONO* (*p54nrb*), *CLTC*, *PSF*, *LUC7L3*, *KHSRP*, *PARP14* and unknown genes on chromosomes 10. Tumors with different specific gene fusions may have slightly different clinical manifestations and morphologic features. In this study, we developed a FISH assay to detect the *TFE3* gene rearrangement for the presence of the 2 most common fusion genes *ASPL-TFE3* and *PRCC-TFE3* in routinely processed archival materials. 10 Xp11 translocation RCCs were detected *TFE3* fusion genes. Cases 1-6 displayed fusion signals of *ASPL-TFE3* and cases 7-10 demonstrated fusion signals of *PRCC-TFE3*. All cases contained a high percentage of cells displaying fusion signals for *ASPL-TFE3* (mean, 45%; range, 35% to 60%) and for *PRCC-TFE3* (mean, 45%; range, 35% to 60%). The sensitivity and specificity were both 100%. The interphase fluorescence in situ hybridization (FISH) assays should enable a more definitive identification of the *ASPL-TFE3* and *PRCC-TFE3* fusion gene in archival material and allow more meaningful clinicopathologic associations to be drawn.

**Keywords:** Xp11 translocation renal cell carcinoma, rearrangement, translocation, *TFE3*, *ASPL*, *PRCC*, FISH, molecular genetics

## Introduction

Xp11 translocation renal cell carcinomas (RCCs) are uncommon renal tumors, characterized by several different translocations involving the *TFE3* gene [1]. In these diseases, the *TFE3* gene is fused by translocation to 1 of several other genes, including *ASPL*, *PRCC*, *NONO* (*p54nrb*), *CLTC*, *PSF*, *LUC7L3*, *KHSRP*, *PARP14* and unknown genes on chromosomes 10 [1-8]. The t(X;17)(p11.2;q25) translocation with *ASPL-TFE3* fusion and the t(X;1)(p11.2;q21) translocation with *PRCC-TFE3* fusion are the 2 most common forms [3, 4, 9, 10]. Tumors with different specific gene fusions may have slightly different clinical manifestations and morphologic features [11]. For example, *ASPL-TFE3*-associated tumors frequently present at an advanced stage and distinctive features including voluminous clear cytoplasm, discrete cell borders, an

alveolar or papillary growth pattern, and psammoma bodies, whereas *PRCC-TFE3*-associated neoplasms tend to possess a nested growth pattern, smaller cells with less abundant cytoplasm, and fewer calcifications [3, 4, 10, 11]. The difference and significance of clinical manifestations from different gene fusions remain to be explored.

Xp11 translocation RCCs can be diagnosed through detection of *TFE3* protein overexpression by immunohistochemistry and *TFE3* gene rearrangements by *TFE3* break-apart fluorescence in situ hybridization (FISH) assays [11]. However, both methods do not provide information as to the specific fusion partners of *TFE3*. Cytogenetic karyotypic analysis and reverse transcriptase polymerase chain reaction (RT-PCR) are 2 common methodologies for identifying the specific gene fusions. Unfortunately,

## Detection of the *ASPL-TFE3* and *PRCC-TFE3* gene fusion in Xp11 RCC

both methods are limited by the need for special handling techniques and are not always easy to apply in routine diagnostic practice [12]. Therefore the specific gene fusions of Xp11 translocation RCCs were rarely confirmed and described in case reports or small series.

In this study, we developed a FISH assay to detect the *TFE3* gene rearrangement for the presence of the 2 most common fusion genes *ASPL-TFE3* and *PRCC-TFE3* in routinely processed archival materials. The FISH assay allows more meaningful clinicopathologic associations to be drawn.

### Materials and methods

#### Case selection

The study was performed on 10 Xp11 translocation RCC cases proven by *TFE3* break-apart FISH assays and/or RT-PCR. The clinicopathologic features, treatments, and follow-up data were recorded (**Table 1**). As control, 5 previously published Xp11 neoplasms with melanocytic differentiation, 16 unrelated RCCs, including 6 clear cell RCCs, 5 papillary RCCs, and 5 chromophobe RCCs, and non-neoplastic renal tissues were entered into the study [1, 13]. Among these Xp11 neoplasms with melanocytic differentiation, 4 cases located in the pancreas, cervix, pelvis and kidney harbor *PSF-TFE3* gene fusions and 1 prostate tumor harbors *NONO-TFE3* gene fusion [1, 13].

#### Detection of the *ASPL-TFE3* and *PRCC-TFE3* fusion genes by reverse transcription polymerase chain reaction

For the *ASPL-TFE3* and *PRCC-TFE3* fusion gene, 2 new primer pairs were designed to detect fusion gene transcripts. The PCR primers were as follows: *ASPL* exon 7 (F), TGCTGCGAGCACACTCAG, *TFE3* exon 6 (R), TCAAGCAGATTCCTGACAC; *PRCC* exon 1 (F), GCCGGAGTTGCATAAGG, *TFE3* exon 6 (R), TCAAGCAGATTCCTGACAC.

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

#### FISH probe design and development

Bacterial artificial chromosome (BAC) clones were selected using the "Clone Central human

BAC Clone Locator" from Empire Genomics ([http://www.empiregenomics.com/Clone\\_Central/gene\\_search](http://www.empiregenomics.com/Clone_Central/gene_search)) as recently described for both fusion and split hybridization experiments [1, 11, 12]. FISH analysis of the *TFE3* split assay was performed on paraffin-embedded tissues as described previously [1, 11, 12]. For the *ASPL-TFE3* and *PRCC-TFE3* fusion assays, the BAC clones RP11-765014 (195 kbp) and RP11-665F9 (176 kbp), located centromeric to the *ASPL* gene locus and the BAC clones RP11-867E4 (221 kbp) and RP11-1150P9 (179 kbp), located centromeric to the *PRCC* gene locus were labeled with 5-fluorescein-dUTP. The BAC clones RP11-416B14 (182 kbp) and RP11-344N17 (202 kbp), located telomeric to the *TFE3* gene locus, were labeled with 5-ROX-dUTP.

#### FISH

A hematoxylin- and eosin-stained slide from each block was examined to identify the areas containing tumor cell clusters for cell counting. Tissue sections that were 3  $\mu$ m in thickness were prepared from buffered formalin-fixed, paraffin-embedded tissue blocks. The deparaffinized tumor tissue on slides was subjected to heat pretreatment (pressure cooking for 10 min at full pressure) in distilled water and then digested using 0.25% pepsin (Sigma, Taufkirchen, Germany) and 0.01 M HCl for 15 min at 37°C. After rinsing twice in 2xSSC for 5 min, the tissues were dehydrated by immersing the slides in 70%, 85%, and 100% ethanol for 1 min each at room temperature and then air-dried. The probes were diluted in *t* DenHyb 2 (Insitus, Albuquerque, NM) in a ratio of 1:25. Slides containing tissue DNA probes (10  $\mu$ l/slide) were co-denatured in an in situ thermocycler (System 1000, Perkin Elmer, Germany) at 83°C for 12 min, annealed at 37°C, and hybridized in a humidified chamber at 37°C overnight. After post-hybridization washing in 0.4xSSC (70°C for 2 min) and 2xSSC (room temperature for 2 min), the slides were coverslipped with 10 ml of 4, 6-diamino-2-phenylindole for counterstaining.

#### FISH evaluation

The method of analysis has been partially described previously [1, 11, 12]. The first step was a split probe assay to detect *TFE3* gene rearrangement. A fused or closely approximat-

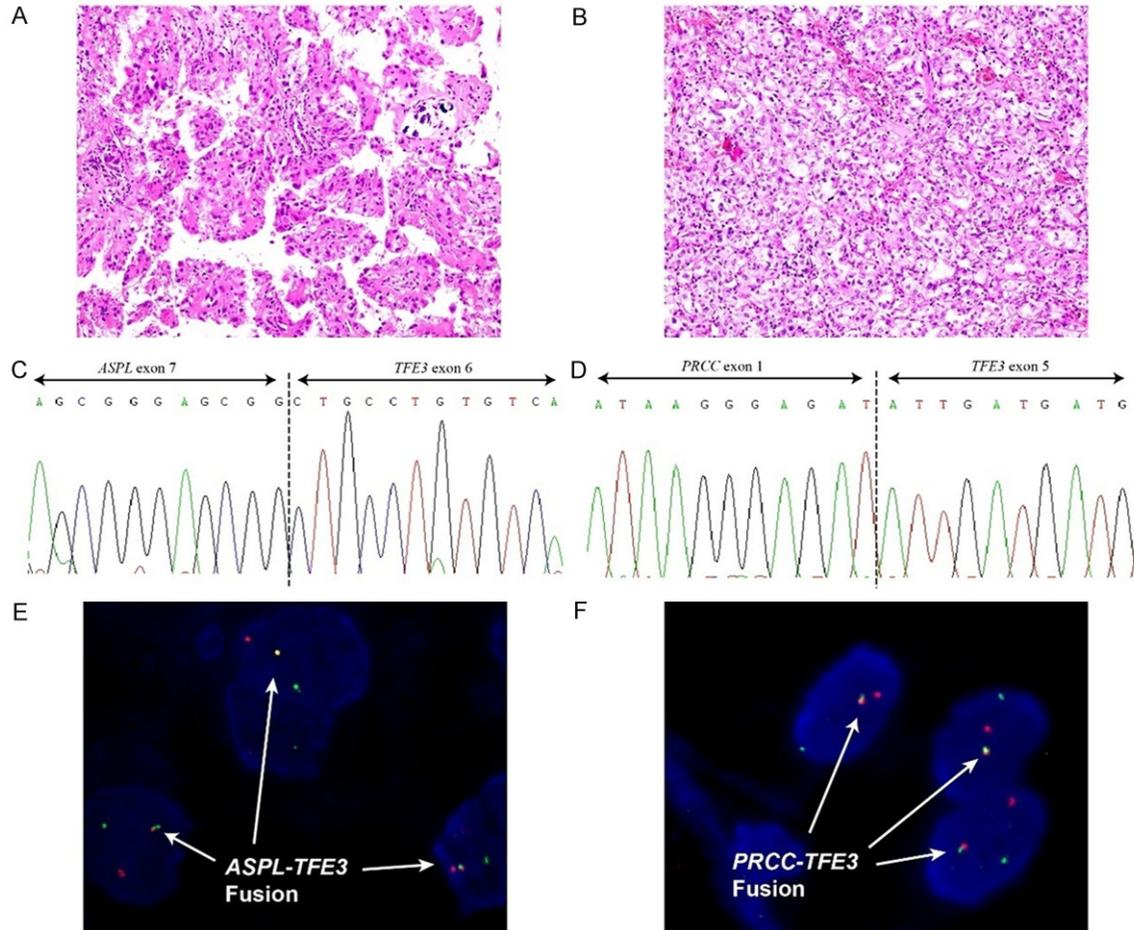
## Detection of the *ASPL-TFE3* and *PRCC-TFE3* gene fusion in Xp11 RCC

**Table 1.** Clinical, morphological, and molecular Data

Case	Consensus Diagnosis	Age/Sex/Tumor Side	Tumor Size	WHO/ISUP	Stage	Pigment	PB	Necrosis	TFE3 Rearrangement		Treatment
									TFE3 split	Fusion partner	
1	Xp11 RCC probable ASPL fusion type	11/F/R	8 cm	3	pT3N0M0	-	+	-	+	ASPL	RN
2	Xp11 RCC probable ASPL fusion type	31/M/L	9 cm	3	pT2N1M0	-	+	-	+	ASPL	RN
3	Xp11 RCC probable ASPL fusion type	27/F/R	10 cm	3	pT4N1M1	-	-	-	+	ASPL	RN
4	Xp11 RCC probable ASPL fusion type	16/F/L	3.5 cm	3	pT1N0M0	-	+	-	+	ASPL	RN
5	Xp11 RCC probable ASPL fusion type	20/M/R	9 cm	3	pT3N1M0	-	+	-	+	ASPL	RN
6	Xp11 RCC probable ASPL fusion type	19/F/L	6.5 cm	3	pT1N1M0	-	+	-	+	ASPL	RN
7	Xp11 RCC probable PRCC fusion type	35/F/R	2 cm	2	pT1N0M0	-	-	-	+	PRCC	PN
8	Xp11 RCC probable PRCC fusion type	23/M/R	8 cm	3	pT2N0M0	-	+	-	+	PRCC	RN
9	Xp11 RCC probable PRCC fusion type	31/F/L	4 cm	2	pT1N0M0	-	-	-	+	PRCC	RN
10	Xp11 RCC probable PRCC fusion type	36/F/L	5 cm	2	pT1N0M0	-	-	-	+	PRCC	RN

RCC, renal cell carcinoma; M, male; F, female; L, left; R, right; RN, radical nephrectomy; PN, partial nephrectomy; PB, psammoma bodies.

## Detection of the *ASPL-TFE3* and *PRCC-TFE3* gene fusion in Xp11 RCC



**Figure 1.** A. The *ASPL-TFE3*-associated Xp11 RCC has nested and papillary architecture, psammoma bodies, and cells with voluminous, and clear to lightly eosinophilic cytoplasm. B. The *PRCC-TFE3*-associated Xp11 RCC is composed of compactly arranged, lightly eosinophilic cells with less abundant cytoplasm and prominent nucleoli. C. The *ASPL-TFE3* gene fusion was confirmed via RT-PCR. D. The *PRCC-TFE3* gene fusion was confirmed via RT-PCR. E. The *ASPL-TFE3* fusion probe assay revealed a single pair of separated green and red signals and an abnormally colocalized signal. The nuclei were stained with DAPI (blue). F. The *PRCC-TFE3* fusion probe assay revealed a single pair of separated green and red signals and an abnormally colocalized signal. The nuclei were stained with DAPI (blue).

ed green-red signal pattern was interpreted as a normal result, whereas a split signal pattern indicated the presence of a *TFE3* fusion.

The second test was a fusion probe assay using a probe telomeric to *TFE3* (5-ROX-dUTP, red) and another probe centromeric to *ASPL* or *PRCC* (5-fluorescein-dUTP, green). Colocalized signal represented a fusion between *ASPL* or *PRCC* and *TFE3*.

The signals were considered to be split when the green and red signals were separated by a distance equal to or greater than two signal diameters. For each case, a minimum of 100 tumor nuclei were examined for the probe sig-

nals via fluorescence microscopy at 1000× magnification. To avoid false-positive interpretations resulting from nuclear truncation, only non-overlapping tumor nuclei were evaluated. Based on the generally accepted guidelines used by all other commercially available break-apart FISH assays and the developed *TFE3* break-apart FISH assays, a positive result was reported when more than 10% of the nuclei in the tumor tissue displayed evidence of *TFE3* gene rearrangement, *ASPL-TFE3* fusion or *PRCC-TFE3* fusion [1, 11, 12].

As negative controls, 5 previously published Xp11 neoplasms with melanocytic differentiation, 16 unrelated RCCs, including 6 clear cell

## Detection of the *ASPL-TFE3* and *PRCC-TFE3* gene fusion in Xp11 RCC

RCCs, 5 papillary RCCs, and 5 chromophobe RCCs, and non-neoplastic renal tissues were evaluated.

### Results

#### Patients

The clinicopathologic characteristics of all 10 Xp11 translocation RCCs are shown in **Table 1**. The patients ranged in age from 11 to 36 years (mean, 24.9 y; median, 25 y). The ratio of male to female patients was 1:2.3. None of the patients had bilateral or multifocal neoplasms. None of the patients reported a personal or family history of tuberous sclerosis complex (TSC), and their disease was not associated with prior chemotherapy, which has been implicated in translocation carcinomas. Nephrectomy was performed at the time of diagnosis for all patients.

#### Morphology

All 10 tumors displayed morphologic features typical of Xp11 translocation RCCs including voluminous cytoplasm, discrete cell borders, alveolar, nested, or papillary architecture, and psammoma bodies. In cases 1-6, the tumor had nested and papillary architecture, psammoma bodies, and cells with voluminous, clear to lightly eosinophilic cytoplasm, and high Fuhrman nuclear grade, typical of an *ASPL-TFE3*-associated neoplasm. Cases 7-10 were composed of compactly arranged lightly eosinophilic cells with less abundant cytoplasm and prominent nucleoli, typical of a *PRCC-TFE3*-associated neoplasm (**Figure 1A** and **1B**).

#### Molecular analysis

Adequate RNA was extracted from formalin-fixed, paraffin-embedded tissues of 7 cases (1, 2, 3, 4, 7, 8, and 9) for RT-PCR analysis. Using primers (*ASPL* exon 7 [F], TGCTGCGAGCACAC-TCAG, *TFE3* exon 6 [R], TCAAGCAGATTCCTGACAC), RT-PCR for detecting fusion gene products revealed a 290-bp *ASPL-TFE3* fusion gene in case 1-4. The chimeric *ASPL-TFE3* transcripts were composed of *ASPL* exon 7 fused with *TFE3* exon 6. Cases 7, 8, and 9 demonstrated an identical *PRCC-TFE3* fusion gene of 153 bp, with the fusion transcript comprising *PRCC* exon 1 fused with *TFE3* exon 5 when using the primers *PRCC* exon 1 (F), GCCGGAGTTGCATAAGG,

*TFE3* exon 6 (R), TCAAGCAGATTCCTGACAC. (**Figure 1C** and **1D**).

#### FISH analysis

On the basis of FISH analysis, all 10 Xp11 translocation RCCs exhibited *TFE3* gene rearrangement. Cases 1-6 displayed fusion signals of *ASPL-TFE3* and cases 7-10 demonstrated fusion signals of *PRCC-TFE3*. All cases contained a high percentage of cells displaying fusion signals for *ASPL-TFE3* (mean, 41%; range, 32% to 58%) and for *PRCC-TFE3* (mean, 43%; range, 30% to 62%) (**Figure 1E** and **1F**).

5 Xp11 neoplasms with melanocytic differentiation, 16 unrelated RCCs (6 clear cell RCCs, 5 papillary RCC, and 5 chromophobe RCCs) and non-neoplastic renal tissues did not exhibit *TFE3* gene rearrangement on the basis of *TFE3* split FISH assay. None of the 21 negative control cases or the non-neoplastic renal tissues displayed positive colabeling on the basis of *ASPL-TFE3* or *PRCC-TFE3* fusion FISH assays.

### Discussion

Xp11 translocation RCCs harbor chromosome translocations that result in 1 of a variety of gene fusions that involve the *TFE3* gene, which maps to the Xp11.2 locus. Reported *TFE3* fusion partners include *ASPL*, *PRCC*, *NONO* (*p54nrb*), *CLTC*, *PSF*, *LUC7L3*, *KHSRP*, *PARP14* and unknown genes on chromosomes 10 [1-8]. It has been increasingly known that tumors with different specific gene fusions may have slightly different clinical manifestations and morphologic features [11]. However, data on the clinicopathologic features of the subtypes of Xp11 translocation RCC associated with specific fusion partners are limited, as demonstration of the fusion partner has typically required fresh tissue for either cytogenetics or reverse transcriptase polymerase chain reaction assays. In this study, we developed a fluorescence in situ hybridization (FISH) assay to evaluate a series of Xp11 translocation RCCs for the presence of the most common gene fusions *ASPL-TFE3* and *PRCC-TFE3*.

All 10 Xp11 translocation RCCs displayed morphologic features typical of Xp11 translocation RCCs. 6 cases demonstrated histology typical of an *ASPL-TFE3*-associated neoplasm. 4 cases

## Detection of the *ASPL-TFE3* and *PRCC-TFE3* gene fusion in Xp11 RCC

(cases 7-10) demonstrated histology typical of a *PRCC-TFE3*-associated neoplasm. Using newly designed primers, RT-PCR for detecting fusion gene products revealed *ASPL-TFE3* fusion genes in case 1-4 and *PRCC-TFE3* fusion genes in case 7-9. The chimeric *ASPL-TFE3* transcripts were composed of *ASPL* exon 7 fused with *TFE3* exon 6. Cases 7, 8, and 9 demonstrated an identical *PRCC-TFE3* fusion gene comprising *PRCC* exon 1 fused with *TFE3* exon 5. The case 10 was failed to be detected for inadequate RNA.

Although RT-PCR can be used for identifying the specific gene fusions, it is limited by the need for special handling techniques and is not always easy to apply in routine diagnostic practice [12]. It is reasonable to conclude that a negative RT-PCR result could be explained by variable breakpoints, by a new fusion partner or by low-quality extracted RNA that prevented the detection of a fusion partner with a long RT-PCR product [1, 12]. Gene fusion detection by RT-PCR is still less reliable than detection by other molecular methods.

In the current study, we developed the *ASPL-TFE3* and *PRCC-TFE3* fusion FISH assays to detect the most common fusion genes of Xp11 translocation RCCs. All 10 Xp11 translocation RCCs were detected *TFE3* fusion genes. Cases 1-6 displayed fusion signals of *ASPL-TFE3* and cases 7-10 demonstrated fusion signals of *PRCC-TFE3*. All cases contained a high percentage of cells displaying fusion signals for *ASPL-TFE3* (mean, 45%; range, 35% to 60%) and for *PRCC-TFE3* (mean, 45%; range, 35% to 60%). None of the 21 negative control cases or the non-neoplastic renal tissues displayed positive colabeling on the basis of *ASPL-TFE3* or *PRCC-TFE3* fusion FISH assays. The sensitivity and specificity were both 100%. The interphase FISH assays should enable a more definitive identification of the *ASPL-TFE3* and *PRCC-TFE3* fusion gene in archival material and should permit further clinicopathologic investigation of these tumors.

### Acknowledgements

This work was supported by National Natural Science Foundation of China (81201187; Zi-Yu Wang); (81472391; 81101933; Qiu Rao), and (81171391; 81372743; Xiao-Jun Zhou).

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Qiu Rao, School of Basic Medical Sciences, Nanjing University of Traditional Chinese Medicine, Nanjing, China; Department of Pathology, Nanjing Jinling Hospital, Nanjing University School of Medicine, Nanjing 210002, Jiangsu, China. Tel: 86-25-80860191; Fax: 86-25-80860191; E-mail: raoqiu1103@126.com

### References

- [1] Rao Q, Shen Q, Xia QY, Wang ZY, Liu B, Shi SS, Shi QL, Yin HL, Wu B, Ye SB, Li L, Chen JY, Pan MH, Li Q, Li R, Wang X, Zhang RS, Yu B, Ma HH, Lu ZF and Zhou XJ. PSF/SFPQ is a very common gene fusion partner in *TFE3* rearrangement-associated perivascular epithelioid cell tumors (PEComas) and melanotic Xp11 translocation renal cancers: clinicopathologic, immunohistochemical, and molecular characteristics suggesting classification as a distinct entity. *Am J Surg Pathol* 2015; 39: 1181-1196.
- [2] Clark J, Lu YJ, Sidhar SK, Parker C, Gill S, Smedley D, Hamoudi R, Linehan WM, Shipley J and Cooper CS. Fusion of splicing factor genes *PSF* and *NonO* (*p54nrb*) to the *TFE3* gene in papillary renal cell carcinoma. *Oncogene* 1997; 15: 2233-2239.
- [3] Argani P, Antonescu CR, Illei PB, Lui MY, Timmons CF, Newbury R, Reuter VE, Garvin AJ, Perez-Atayde AR, Fletcher JA, Beckwith JB, Bridge JA and Ladanyi M. Primary renal neoplasms with the *ASPL-TFE3* gene fusion of alveolar soft part sarcoma: a distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. *Am J Pathol* 2001; 159: 179-192.
- [4] Argani P, Antonescu CR, Couturier J, Fournet JC, Sciort R, Debiec-Rychter M, Hutchinson B, Reuter VE, Boccon-Gibod L, Timmons C, Hafez N and 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, Lui MY, Couturier J, Bouvier R, Fournet JC and Ladanyi M. A novel *CLTC-TFE3* gene fusion in pediatric renal adenocarcinoma with *t(X;17)(p11.2;q23)*. *Oncogene* 2003; 22: 5374-5378.
- [6] 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 and Ladanyi M. Xp11 translocation renal cell carcinoma in adults: expanded clinical,

## Detection of the *ASPL-TFE3* and *PRCC-TFE3* gene fusion in Xp11 RCC

- pathologic, and genetic spectrum. *Am J Surg Pathol* 2007; 31: 1149-1160.
- [7] Malouf GG, Su X, Yao H, Gao J, Xiong L, He Q, Comperat E, Couturier J, Molinie V, Escudier B, Camparo P, Doss DJ, Thompson EJ, Khayat D, Wood CG, Yu W, Teh BT, Weinstein J and Tannir NM. Next-generation sequencing of translocation renal cell carcinoma reveals novel RNA splicing partners and frequent mutations of chromatin-remodeling genes. *Clin Cancer Res* 2014; 20: 4129-4140.
- [8] Huang W, Goldfischer M, Babyeva S, Mao Y, Volyanskyy K, Dimitrova N, Fallon JT and Zhong M. Identification of a novel *PARP14-TFE3* gene fusion from 10-year-old FFPE tissue by RNA-seq. *Genes Chromosomes Cancer* 2015; [Epub ahead of print].
- [9] Qiu R, Bing G and Zhou XJ. Xp11.2 Translocation renal cell carcinomas have a poorer prognosis than non-Xp11.2 translocation carcinomas in children and young adults: a meta-analysis. *Int J Surg Pathol* 2010; 18: 458-464.
- [10] Rao Q, Chen JY, Wang JD, Ma HH, Zhou HB, Lu ZF and Zhou XJ. Renal cell carcinoma in children and young adults: clinicopathological, immunohistochemical, and *VHL* gene analysis of 46 cases with follow-up. *Int J Surg Pathol* 2011; 19: 170-179.
- [11] Rao Q, Williamson SR, Zhang S, Eble JN, Grignon DJ, Wang M, Zhou XJ, Huang W, Tan PH, MacLennan GT and 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.
- [12] Rao Q, Liu B, Cheng L, Zhu Y, Shi QL, Wu B, Jiang SJ, Wang Y, Wang X, Yu B, Zhang RS, Ma HH, Lu ZF, Tu P, Wang JD and Zhou XJ. Renal Cell Carcinomas With t(6;11)(p21;q12): A Clinicopathologic Study Emphasizing Unusual Morphology, Novel Alpha-*TFEB* Gene Fusion Point, Immunobiomarkers, and Ultrastructural Features, As Well As Detection of the Gene Fusion by Fluorescence In Situ Hybridization. *Am J Surg Pathol* 2012; 36: 1327-1338.
- [13] Wang XT, Xia QY, Ni H, Wang ZY, Ye SB, Li R, Wang X, Lv JH, Shi SS, Ma HH, Lu ZF, Shen Q, Zhou XJ and Rao Q. Xp11 Neoplasm With Melanocytic Differentiation of the Prostate Harboring the Novel *NONO-TFE3* Gene fusion: Report of a Unique Case Expanding the Gene Fusion Spectrum. *Histopathology* 2016; [Epub ahead of print].