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

Zi-Yu Wang¹, Qiu-Yuan Xia², Shan-Shan Shi², Sheng-Bing Ye², Rui Li², Heng-Hui Ma², Zhen-Feng Lu², Xiao-Jun Zhou², Qiu Rao¹²

¹School of Basic Medical Sciences, Nanjing University of Traditional Chinese Medicine, Nanjing, China; ²Department 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), TCAAGCAGATTCCGACAC; PRCC exon 1 (F), GCCGGAGTTGCAATAGG, TFE3 exon 6 (R), TCAAGCAGATTCCGACAC.

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) 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 μm 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, Tauferkchen, 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 μl/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-
Table 1. Clinical, morphological, and molecular Data

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

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

A normal 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 signals 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

![Figure 1](image-url)
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], TGCTGGAGCACACAGTGTTCAAG, TFE3 exon 6 [R], TCAAGCAGATTCCCTGACAC), 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), GCGGAGTTGCAAGG, TFE3 exon 6 (R), TCAAGCAGATTCCCTGACAC. (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).

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


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


