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


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The unbalanced translocation, der(17)(X;17)(p11.2;q25), is characteristic of alveolar soft part sarcoma (ASPS). We have recently shown that this translocation fuses the TFE3 transcription factor gene at Xp11.2 to ASPL, a novel gene at 17q25. We describe herein eight morphologically distinctive renal tumors occurring in young people that bear the identical ASPL-TFE3 fusion transcript as ASPS, with the distinction that the t(X;17) translocation is cytogenetically balanced in these renal tumors. A relationship between these renal tumors and ASPS was initially suggested by the cytogenetic finding of a balanced t(X;17)(p11.2;q25) in two of the cases, and the ASPL-TFE3 fusion transcripts were then confirmed by reverse transcriptase-polymerase chain reaction. The morphology of these eight ASPL-TFE3 fusion-positive renal tumors, although overlapping in some aspects that of classic ASPS, more closely resembles renal cell carcinoma (RCC), which was the a priori diagnosis in all cases. These tumors demonstrate nested and pseudodopapillary patterns of growth, psammomatous calcifications, and epithelioid cells with abundant clear cytoplasm and well-defined cell borders. By immunohistochemistry, four tumors were negative for all epithelial markers tested, whereas four were focally positive for cytokeratin and two were reactive for epithelial membrane antigen (EMA) (one diffusely, one focally). Electron microscopy of six tumors demonstrated a combination of ASPS-like features (dense granules in four cases, rhomboid crystals in two cases) and epithelial features (cell junctions in six cases, microvilli and true glandular lumens in three cases). Overall, although seven of eight tumors demonstrated at least focal epithelial features by electron microscopy or immunohistochemistry, the degree and extent of epithelial differentiation was notably less than expected for typical RCC. We confirmed the balanced nature of the t(X;17) translocation by fluorescence in situ hybridization in all seven renal tumors thus analyzed, which contrasts sharply with the unbalanced nature of the translocation in ASPS. In summary, a subset of tumors previously considered to be RCC in young people are in fact genetically related to ASPS, although their distinctive morphological and genetic features justify their classification as a distinctive neoplastic entity. Finally, the finding of distinctive tumors being associated with balanced and unbalanced forms of the same translocation is to our knowledge, unprecedented. (Am J Pathol 2001, 159:179–192)

We have recently identified the gene fusion resulting from the chromosome translocation that is characteristic of alveolar soft part sarcoma (ASPS), der(17)(X;17)(p11.2;q25), by fluorescence in situ hybridization. We have confirmed this finding in a subset of renal tumors that were initially diagnosed as RCC. The morphology and immunohistochemical features of these tumors suggest a relationship to ASPS, although they lack the distinctive cytogenetic features of this tumor.

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q25). This unbalanced translocation results in the fusion of the TFE3 gene on Xp11.2, a member of the basic-helix-loop-helix family of transcription factors, to a novel gene named ASPL on 17q25. The resulting fusion gene encodes chimeric ASPL-TFE3 RNA transcripts. Two mutually exclusive types of fusion products, designated type 1 and type 2, are observed. They differ in their exon composition, reflecting, respectively, the exclusion or inclusion of TFE3 exon 3 in the ASPL-TFE3 fusion transcript. TFE3 was previously found to be rearranged by specific reciprocal translocations involving Xp11.2 in a subset of pediatric renal cell carcinomas (RCCs). In most of these cases, the translocation partner is a novel gene designated PRCC at 1q21.2, whereas in others, TFE3 fuses instead with the splicing factor genes, PSF at 1p34 or NonO at Xq12. ASPL shows no sequence similarities to PRCC, PSF, or NonO.

Of note, other variant translocations involving Xp11.2 have been identified in tumors reported as RCCs. These include four published reports of pediatric renal carcinomas with a balanced t(X;17)(p11.2;q25), the breakpoints of which are cytogenetically identical to the translocation now recognized to be characteristic of ASPS. Intriguingly, ASPS is well known to be capable of mimicking RCC morphologically, in that both tumors feature nested and alveolar patterns of growth bounded by prominent sinusoidal vasculature, and both contain polygonal cells with clear-eosinophilic cytoplasm and distinct borders. Given these morphological similarities and the cytogenetic findings, we hypothesized that such renal tumors might in fact be related to ASPS.

Therefore, we have reviewed several of these reported carcinomas, along with cases coded as RCC in the files of two of the authors (JBB, VER), with the aim of identifying tumors bearing the ASPL-TFE3 gene fusion characteristic of ASPS. We report herein the clinical, pathological, and genetic features of eight primary renal neoplasms in which we have identified the ASPL-TFE3 fusion.

Materials and Methods

Study Group

We initially searched the files of the National Wilms Tumor Study Pathology Center (NWTSPC) and the affiliated consultation files of JBB for cases of carcinoma with available cytogenetic results. Two cases with a documented t(X;17)(p11.2;q25) were identified; one of these cases was one of four previously published cytogenetic reports of RCC with a t(X;17), as noted above. Fresh frozen tissue suitable for RNA extraction for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was available from both tumors. Based on the morphological features of these two tumors that were noted to be distinctive and virtually identical (see Results), we reviewed all renal carcinomas in the files of JBB (~80 cases) and all cases classified as either clear cell RCC, papillary RCC, or unclassifiable RCC in the files of Memorial Sloan-Kettering Cancer Center (MSKCC) from 1990 to 1998 (~400 cases, compiled by VER) with the goal of identifying morphologically similar cases for which fresh frozen tissue was available. Cases 3 to 7 were identified in this search. Of note, cases 4 and 7 were initially reported as pediatric papillary renal carcinomas with voluminous cytoplasm, with the former bearing a translocation originally reported as der(X)t(X;7)(p11.2;q11) (see Results). Case 8 is an additional anecdotal case identified recently by one of the authors (AJG). Clinical follow-up was obtained from the referring pathologist in each case.

Immunohistochemistry

After morphological identification of the cases, immunohistochemical labeling was performed on all eight cases at The Johns Hopkins Hospital on a single formalin-fixed, paraffin-embedded tissue block from each case. Briefly, 4-μm sections were deparaffinized in xylene for 30 minutes and rehydrated using graded ethanol concentrations. Antigen retrieval was performed using either protease digestion or steaming. Immunohistochemical labeling using the avidin-biotin peroxidase complex technique and 3', 3'-diaminobenzidine as chromogen was performed with the automated Biotek-1000 staining system (Ventana/Biotek Solutions, Inc., Tucson, AZ). The antibodies used, vendors, pretreatments, and dilutions were as follows: EMA (DAKO, Carpinteria, CA; steam, 1:1000), desmin (DAKO; steam, 1:20,000), S100 protein (DAKO; steam, 1:6000), cytokeratin 7 (DAKO; protease, 1:50), vimentin (Zymed, San Francisco, CA; steam, 1:100), cytokeratin AE1/AE3 (Boehringer Mannheim, Indianapolis, IN; protease, 1:2000), cytokeratin Cam5.2 (Becton Dickinson, San Jose, CA; protease, prediluted), and HMB45 (DAKO; steam, 1:125). The rationale for applying this panel of antibodies is as follows. The combination of cytokeratin cocktail AE1/AE3 (which recognizes cytokeratins 1 to 8, 10, 14/15, 16, and 19) and cytokeratin cocktail Cam5.2 (which recognizes cytokeratins 8, 18, and 19) was chosen because it is a highly sensitive and well-recognized screen for cytokeratin expression. Stains for cytokeratin 7 and EMA were performed because of their known reactivity with papillary RCC. Stains for vimentin were performed chiefly for the purpose of documenting the immunoreactivity of the tissue section used, as evidenced by strong immunoreactivity of the intratumoral capillaries. Stains for S100 protein, HMB45 and desmin were performed to exclude melanoma, angiomylipoma, and myogenic sarcomas. Stains for EMA and cytokeratin AE1/AE3 were repeated on all cases using the DAKO Envision system to eliminate the possibility of biotin-related artifactual staining.

Electron Microscopy

Ultrastructural studies were performed at MSKCC (by CRA) on representative tumor tissue obtained from either paraffin blocks or from fresh tumor. Tissue removed from the paraffin block was cut into small pieces. The pieces were soaked in xylene, with several changes, for 1 week. After this, the pieces were placed in 100% alcohol, then rehydrated through graded alcohols (100, 95, 80, 70, to
50%) for at least one-half hour each. Tissue was then soaked in buffer for one-half hour or until the tissue was osmicated. Representative fresh tumor tissue was fixed in 2% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in epoxy resin using standard procedures. In each case, thick sections were cut and stained with toluidine blue from four embedded tissue blocks to select blocks suitable for ultrastructural evaluation. Thin sections were stained with uranyl acetate followed by lead citrate and examined with a Philips EM401 electron microscope (Eindhoven, The Netherlands).

Molecular Analysis

Molecular studies were performed at MSKCC on snap-frozen tissue in seven cases, or frozen short-term primary culture (one case). RNA extraction was performed from frozen tissue using a standard organic extraction method (Trizol; Life Technologies, Inc., Friendsworth, TX).18 To assess the adequacy of RNA for analysis, RT-PCR was performed using primers spanning an intron of the ubiquitously expressed phosphoglycerate kinase (PGK) gene, resulting in amplification of a 247-bp fragment, as described in detail elsewhere.19 RNA samples in which the PGK product could not be demonstrated were considered inadequate for ASPL-TFE3 analysis. Negative controls lacking RNA were included in all RT-PCR assays. The amplified fragments were identified by their size on agarose gel electrophoresis. The specificity of all positive results was further confirmed by negative controls containing the target RNA but lacking RT.

To detect the presence of an ASPL-TFE3 fusion transcript, we performed RT-PCR using a forward primer from ASPL (AAAGAAGTCCAAAGTCGGCCA) and a TFE3 exon 4 reverse primer (CGTTGTAGTTGGCACTCA), as previously described.1 RT-PCR for the reciprocal fusion product, TFE3-ASPL, was performed using an ASPL reverse primer (CACCGTCAGCTCAAAGAACTC) and a TFE3 forward primer appropriate to the type of ASPL-TFE3 rearrangement in each case: for cases with a type 1 ASPL-TFE3 fusion, a TFE3 exon 3 forward primer (TGATGATGTGATGAGATC), and in cases with a type 2 ASPL-TFE3 fusion, a more upstream TFE3 primer (GCTCAAAAGCCACAACCTTAC). The ASPL cDNA sequence and junctional sequences of both types of ASPL-TFE3 fusion transcripts have been deposited in GenBank (accession numbers AF324219, AY034077, AY034078).

Fluorescence in Situ Hybridization (FISH)

FISH studies were performed at MSKCC on cytological touch preparations of frozen tissue of nine ASPS specimens (including cases ASPS-1 to 7, and ASPS-11, from our previous study,1 and an additional case, ASPS-13) and six renal tumors (cases 1, 2, 3, 5, 6, and 8), and on cells of a primary culture of one additional renal tumor (case 4). We used two probes in a bicolor FISH assay: the RPC11 human bacterial artificial chromosome (BAC) clone 525L23 (Research Genetics, Huntsville, AL) in combination with the centromere 17-specific alphoid sequence probe [CEP 17 (Vysis, Downer’s Grove, IL)]. On the current version of the human genome sequence GenBank Map (at www.ncbi.nlm.nih.gov), BAC 525L23 maps to 17q25.3 telomeric to BAC RPC11–498C9 that by our previous sequence analysis is known to contain ASPL.1 The map location of BAC 525L23 telomeric to ASPL is confirmed by our FISH results in ASPS cases (see below). One μg of each DNA probe was directly labeled with SpectrumOrange or SpectrumGreen using a nick translation kit according to the manufacturer’s instructions (Vysis). The labeled DNA was then co-precipitated for annealing purposes with 2 μg of Cot-1 DNA (sonicated total human DNA). The chromosomal and telomeric location of the 525L23 BAC probe was first confirmed by hybridizing to metephase spreads of normal human lymphocytes in a bicolor assay with CEP17. Interphase FISH studies on the ASPS and renal cases were then performed with the same combination of the CEP17 and 525L23 BAC probes. Bicolor FISH studies, whether metaphase or interphase, were performed as follows. The slides to be hybridized were pretreated with collagenase H (0.01% in Kreb’s ringer at 37°C for 15 minutes) followed by formaldehyde postfixation [2% (v/v) in MgCl2 phosphate-buffered saline (PBS) for 10 minutes at room temperature], and followed by incubation in 2 mg/ml glycine-PBS. The slides were then prehybridized in 2× standard saline citrate (37°C for 30 minutes) and air-dried. Subsequently, the slides were denatured in 70% formamide, 2× standard saline citrate, pH 7.3, at 73 to 74°C for 6 minutes and placed immediately into cold 70% EtOH, followed by a cold ethanol series of 80, 90, and 100%. The slides were then placed on a slide warmer for 1 minute at 45°C before hybridization. The probes were denatured at 73 to 74°C for 5 minutes. The cells and probes were sealed under an 18-×18-mm coverslip and incubated for 16 to 18 hours at 37°C in a humidity chamber. Images were prepared using the Applied Image Analysis System (Applied Imaging, Pittsburgh, PA). The number of hybridization signals for each probe was assessed in a minimum of 200 interphase nuclei with strong and well-delineated signals. As negative controls, normal peripheral-blood lymphocytes were simultaneously hybridized with these probes.

Results

Clinical Features

The features of the eight cases are summarized in Table 1. There were five females and three males. The patients ranged from 17 months to 17 years of age. Three of the children with smaller tumors (cases 1, 3, and 8) presented with hematuria, whereas another patient with a small tumor (case 4) presented with a urinary tract infection. Two of the patients with larger tumors (cases 2 and 5) presented with abdominal masses. Patient 7 presented with flank pain.
Gross Pathological Features

Primary tumor diameters ranged from 2.3 to 14 cm. On cut surface, the tumors appeared to be soft and well circumscribed, with four described as tan, two as yellow, and one as pink. Five tumors were noted to be necrotic, whereas three demonstrated hemorrhagic foci. Five of the eight tumors demonstrated capsular penetration. No tumor involved the renal vein grossly. One tumor (case 7) arose in a kidney bearing a duplicated collecting system. No other abnormalities of the nonneoplastic kidneys were noted.

Light Microscopic Features

The morphological features of these eight tumors were similar, and therefore they are described together. All tumors appeared well circumscribed but unencapsulated at low power. Their basic architecture was organoid, with variably sized nests of tumor cells separated by delicate, thin fibrovascular septa (Figure 1). In some foci, the nests were solid and the fibrovascular septa were thin and inapparent giving rise to a sheet-like growth pattern (Figure 2). Although cells in the larger nests were focally dyscohesive, giving rise to the alveolar pattern, the septal vessels generally did not demonstrate the sinusoidal dilation usually associated with ASPS. In seven of the eight cases, foci of hemorrhage within the nests yielded a morphological appearance typically associated with clear cell RCC.20

The septa delineating the larger nests often did not connect, creating a pseudopapillary pattern as tumor cells clung to the branching fibrovascular septa (Figure 3). Unlike true papillae, these pseudopapillae generally demonstrated an irregular luminal border, and the nuclei were not aligned abluminally. Alternatively, large nests contained cross-sections of fibrovascular septa, to which occasional tumor cells clung. Such fibrovascular septa were often hyaline, and were associated with psammomatous calcifications in every case. Case 6 focally demonstrated trabecular and cribriform growth patterns with smooth luminal borders and extensively branching papillae, highly suggestive of true epithelial differentiation (Figure 4).

On high-power examination, tumor cells contained voluminous cytoplasm that was sharply demarcated by distinct cell borders (Figure 5). The cytoplasm was usually clear or finely granular; however, a subset of tumor cells...
Table 1. Continued

<table>
<thead>
<tr>
<th>Electron microscopy findings*</th>
<th>Cytogenetic t(X;17)</th>
<th>t(X;17) fusion transcripts by RT-PCR</th>
<th>17qter copy number by FISH</th>
<th>Follow-up</th>
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<tr>
<td>ASPS crystals, dense granules, CJ with lumens and microvilli, BM</td>
<td>t(X;17) (p11.2;q25)</td>
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<tr>
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<td>t(X;17) (p11.2;q25)</td>
<td>Type 1</td>
<td>+</td>
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<tr>
<td>ND</td>
<td>Failed</td>
<td>Type 1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
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<td>der(X)t(X;7) (p11; p11), add(17) (q24–25)$</td>
<td>Type 1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Dense granules, CJ</td>
<td>ND</td>
<td>Type 1</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>Dense granules with early crystallization, rare CJ</td>
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<td>Glycogen, fat, CJ with lumens and microvilli</td>
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<td>Type 1</td>
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* Dense granules refers to granules similar in size and shape to those seen in ASPS (see text).
† Case previously reported as reference 7.
‡ Cases previously reported within reference 13.
§ Probable complex t(X;17), see text for complete karyotype.
¶ Out-of-frame fusion transcript (see text).
$ Unresectable retroperitoneal metastasis.
f, focal; d, diffuse; CAM, cam5.2 antibody; CJ, cell junctions; BM, basement membrane material; ND, not done; AML, acute myelogenous leukemia; NED, no evidence of disease; DOD, died of disease; AWD, alive with disease; AJCC, American Joint Committee on Cancer, 1997 Revision; NWTS, National Wilms Tumor Study.

Figure 2. Low-power view of case 2 showing solid, sheet-like growth pattern where the alveolar septa do not connect (H&E, original magnification, ×100).

Figure 3. Intermediate-power view of case 1 showing pseudopapillary growth pattern and psammomatous calcification that occurs in hyaline cores of small pseudopapillae (H&E, original magnification, ×160).
was densely granular and overtly eosinophilic. Larger, amphophilic cytoplasmic granules were also identified in each case. Nuclei were eccentrically placed within the cells, and were somewhat rounded but had irregular borders on closer inspection. The chromatin was vesicular, with a single prominent nucleolus. Mitoses were extremely rare. In each tumor, periodic acid-Schiff (PAS) stains demonstrated fine granular cytoplasmic positivity that primarily disappeared with diastase treatment, consistent with glycogen. After diastase treatment, most of the tumors demonstrated scattered PAS-positive, diastase-resistant cytoplasmic granules; these were most prominent in case 4. However, none of the tumors demonstrated the well-formed PAS-positive, diastase-resistant intracytoplasmic crystals that are characteristic of ASPS.

These tumors tended to present at high stage. By NWTS-5 staging criteria, one tumor presented at stage 4 (because of bony metastasis), six at stage 3 (four because of lymph node metastasis, one because of piecemeal removal of tumor, one because of tumor unresectability), and one was stage 1. Using 1997 American Joint Committee on Cancer criteria, three tumors were stage IV, four tumors were stage III, and one tumor was stage I. All eight tumors demonstrated intrarenal vascular invasion. Perirenal (renal sinus) lymph nodes contained metastases in six of the seven cases in which nodes were identified on pathological examination.

**Immunohistochemical Features**

Vimentin highlighted the capillaries of the fibrovascular septa of all cases. Vimentin was negative in five of the
tumors, with only focal labeling of tumor cells in cases 4, 5, and 7. Cytokeratin antibody Cam5.2 labeled two tumors focally, whereas cytokeratin antibody AE1/3 labeled three tumors focally. Antibody to EMA focally labeled two of the latter tumors, and diffusely labeled another (case 6) that had shown prominent epithelial growth patterns (Figure 4B). The two tumors (cases 6 and 8) positive for EMA also labeled focally for S100 protein, whereas none of the other tumors did. The cytokeratin and EMA stains highlighted native renal tubules in contact with the nonreactive tumors, which emphasized their unencapsulated nature (Figure 6, A and B). None of the eight tumors labeled for desmin or HMB45.

Ultrastructural Features

Ultrastructural examination was performed on six cases, five on glutaraldehyde-fixed tissue, and a sixth on formalin-fixed, paraffin-embedded tissue. Four tumors demonstrated a distinctive combination of ASPS-like and epithelial features. These tumors all demonstrated abundant electron-dense granules that were similar in size and shape to those seen in ASPS and are not typical of any primary renal tumor. One of these four tumors, case 1, revealed well-formed rhomboid crystals, 270- to 276-nm wide in cross-section, composed of fibers with a periodicity of 10 nm and a diameter of 4.5 to 5.0 nm (Figure 7). These features are characteristic of the crystals of ASPS.21 In one other case (case 6), partial or incipient crystallization typical of ASPS crystals was noted (Figure 8). However, each of the four cases also demonstrated abundant well-formed cell junctions, more than are typically seen in soft tissue ASPS, and suggestive of epithelial differentiation. Case 1 demonstrated more definitive epithelial differentiation, as it revealed prominent basement membrane, numerous cell junctions, and well-formed glandular lumens with microvilli (Figure 7). Case 1 therefore demonstrates features that are characteristic of ASPS and adenocarcinoma simultaneously. The two other tumors, cases 7 and 8, demonstrated features more typical of conventional RCC (intracellular glycogen, and fat, well-formed glandular lumens with microvilli) without the crystals or dense granules typical of ASPS.

Cytogenetic Features

The partial karyotype of case 1 is shown in Figure 9. The karyotype of case 2 has been previously reported.7 Cytogenetic analysis was not performed in case 3, 5, 6, 7, or 8. Case 4 was previously reported in brief as showing a der(X)t(X; 7)(p11;p11).13 In fact, the initial karyotype was noted to be complex, and initially described as an 46,X,der(X)t(X;7)(p11; p12),del(1)(p34),add(1)(p34),+5,−6,add(7)(p11.2),+15, del(16)(p11.2),−17,add(20)(q13). Re-evaluation of this karyotype now suggests that it is better described as: 46,X,der(X)t(X; 7)(p11;p11),del(1)(p34),add(1)(p34),+5,−6,add(7)(p11.2), del(16)(p11.2),+17,add(17)(q24-25),add(20)(q13). Overall, this set of abnormal chromosomes could be explained by a complex t(X;17)(p11;q25), with translocation of Xp11 to 17q25, but of 17q25 to another chromosome. Thus, although nonreciprocal, the rearrangements in this case may nonetheless be genomically balanced in terms of chromosomes X and 17.
The further molecular and FISH analyses in this case support this interpretation (see below).

**Molecular Detection of the ASPL-TFE3 Fusion Transcript**

RT-PCR performed on RNA extracted from frozen tumor (cases 1 to 3 and 5 to 8) or frozen short-term culture (case 4) identified a specific ASPL-TFE3 fusion product in all eight cases, consistent with the presence of a t(X;17)(p11.2;q25) (Figure 10). Two mutually exclusive types of ASPL-TFE3 fusion transcripts were observed, corresponding to the two previously described types of ASPL-TFE3 fusion transcripts. Cases 2 to 8 contained the type...
1 product, a fusion of ASPL to TFE3 exon 4, whereas case 1 showed a type 2 product, a fusion of ASPL to TFE3 exon 3. We tested seven other unrelated renal tumors (three clear cell carcinomas, two chromophobe carcinomas, one papillary carcinoma, one angiomyolipoma) as well as the K562 leukemia cell line by RT-PCR for ASPL-TFE3 and they were all negative (results not shown).

Molecular Detection of the Reciprocal TFE3-ASPL Fusion Transcript

Because the t(X;17) in cases 1 and 2 appeared cytogenetically balanced, it should also lead to the formation of a TFE3-ASPL fusion gene. Therefore, we also tested all of the present cases for the reciprocal fusion product, TFE3-ASPL. Using appropriate TFE3 forward and ASPL reverse primers (see Materials and Methods), we detected a TFE3-ASPL fusion transcript in five of eight cases (Figure 11 and Table 1), confirming that the rearrangement between the two genes was reciprocal in these cases. We performed direct sequencing of the TFE3-ASPL RT-PCR products to verify their fusion points. Cases 2, 5, and 8 showed the in-frame TFE3 exon 3 to ASPL fusion expected from a reciprocal type 1 rearrangement (Figure 11). Case 1, which showed a type 2 rearrangement, also contained an in-frame TFE3-ASPL fusion transcript, but the TFE3 fusion point was within TFE3 exon 2 (Figure 12). Finally, case 6, which had a type 1 ASPL-TFE3 fusion, contained a reciprocal fusion in which a 113-nucleotide ALU-family-repeat sequence from approximately the midpoint of intron 3 of TFE3 was inserted between TFE3 exon 3 and ASPL, disrupting the reading frame (Figure 12), leading to premature termination within the portion encoded by the ASPL. This unusual product was not obtained when RT was omitted (result not shown), confirming that it was originating from cDNA and not...
genomic DNA. Thus, although five of the eight cases showed evidence of expression of a TFE3-ASPL fusion transcript arising from a reciprocal rearrangement between ASPL and TFE3, only four of these cases contained in-frame TFE3-ASPL transcripts, capable of encoding potentially functional proteins.

**FISH Analysis of 17q25.3 Copy Number Telomeric to ASPL**

Although the presence of a TFE3-ASPL fusion gene could not be confirmed by RT-PCR in three of the eight cases (see above), we hypothesized that such cases may nonetheless contain a balanced rearrangement that is not leading to the expression of a TFE3-ASPL fusion RNA detectable by RT-PCR, either because the TFE3-ASPL fusion gene is not actively transcribed, or because they contain a more complex rearrangement (eg, three-way) that would produce an ASPL-TFE3 fusion gene but not a TFE3-ASPL fusion gene. To test this hypothesis, we performed FISH to assess the copy number of the portion of 17q25.3 telomeric to the breakpoint in ASPL, reasoning that a balanced rearrangement (in the renal tumors) would result in retention of both copies of this region, whereas an unbalanced translocation (as in ASPS) would result in consistent loss of one copy. We used a bicolor FISH assay on interphase nuclei from frozen material available in seven of our renal cases, and in nine ASPS cases, using as a probe for 17q25.3 the BAC 525L23, known to map telomeric to ASPL (see Materials and Methods), in combination with the centromere 17-specific probe, CEP 17. All seven renal cases tested showed retention of both copies of BAC 525L23 (Table 1), whereas eight of nine ASPS showed loss of one copy (Figure 13). The seven renal cases tested included two of the three cases above in which no TFE3-ASPL fusion RNA was detected by RT-PCR. One of these two cases (case 4), had been karyotyped and in fact contained a more complex rearrangement (eg, three-way) that produced a ASPL-TFE3 fusion gene but would not be expected to generate a TFE3-ASPL fusion gene. Thus, the cytogenetic, ASPL-TFE3 RT-PCR, TFE3-ASPL RT-PCR, and FISH data in this case were entirely consistent with each other. The second of these two cases (case 3) had no cytogenetic data available, but the finding that both copies of BAC 525L23 were retained in this case, as well as all other cases tested, supports our hypothesis that the t(X;17) in these renal tumors is consistently genomically balanced, even if not necessarily simply reciprocal. Interestingly, the single case of ASPS without loss of BAC 525L23 was also the only case that we previously found to contain a TFE3-ASPL fusion transcript (case ASPS-7 from our
previous study\(^1\), further cross-validating the different approaches.

**Clinical Follow-Up**

Four patients (cases 1, 3, 4, and 5) are alive with no evidence of disease at 4 years, 7 years, 2.5 years, and 1.1 years, respectively. Of note, patients 1 and 3 are known to have not received any adjuvant therapy after surgery, whereas information regarding therapy is not available for patients 4 and 5. Patient 7 developed a pulmonary metastasis 15 months after surgery. Her unresectable retroperitoneal tumor showed no evidence of response to interleukin-2 therapy. Patient 2 is alive with stable retroperitoneal disease 12 years after surgery. Therapy with trimetrexate after the initial incomplete resection had stabilized the residual tumor, whereas \(\alpha\)-interferon therapy did not have significant effect. Despite adjuvant chemotherapy, patient 6 died of progressive bony and lymph node metastases 2 years after surgery. At time of writing, patient 8 was newly diagnosed, with only 2 months of follow-up and no evidence of disease.

**Discussion**

We describe herein eight morphologically distinctive primary renal tumors of young patients that are genetically related to but distinct from soft tissue ASPS. All eight of these tumors had been initially classified as RCCs, as their morphology is consistent with that diagnosis. Retrospectively, we suspected that two of these tumors were genetically related to ASPS because they were characterized cytogenetically by the chromosome translocation, t(X;17)(p11.2;q25). An unbalanced chromosome translocation with cytogenetically identical breakpoints has been characterized recently as a hallmark of ASPS.\(^1\) Prompted by the cytogenetic findings, we analyzed these renal tumors for the ASPL-TFE3 fusion by RT-PCR. Indeed, ASPL-TFE3 gene fusions were demonstrated in RNAs extracted from frozen material from each of the eight renal tumors. However, using FISH probes for the centromere and long arm telomere of chromosome 17 and RT-PCR for the chimeric RNA encoded by a reciprocal TFE3-ASPL fusion gene, we were able to document a unique and intriguing distinction between the t(X;17) in these renal tumors and in soft tissue ASPS. The t(X;17) translocation proved to be balanced in all seven renal tumors tested, in sharp contrast to soft tissue ASPS. The t(X;17) identified in the single section from case 1 stained with PAS-diastase. Finally, the tendency of the ASPL-TFE3 renal tumors to present at high stage is also reminiscent of the behavior of ASPS, as is the indolent clinical course experienced by patient 2.

However, aside from their renal location, these tumors demonstrate several features that are more typical of RCC than of soft tissue ASPS. First, these renal tumors frequently spread to lymph nodes, which are commonly involved by RCC only rarely involved by usual soft tissue ASPS.\(^9–12\) Second, the consistent presence of psammoma bodies, clear cell cytology, and pseudopapillary architecture give these lesions an appearance that is
distinct from soft tissue ASPS and highly suggestive of carcinoma. Along these lines, only rare soft tissue ASPS have been reported to contain psammomatous calcifications,24,25 and most are composed of eosinophilic cells in well-defined nests.26 Third, a higher percentage (four of eight, or 50%) of ASPL-TFE3 renal tumors than soft tissue ASPS (4%) labeled, albeit focally, for cytokeratin, which is present in >85% of RCCs.12 Finally, three of the ASPL-TFE3 renal tumors (cases 1, 7, and 8) did show definitive evidence of epithelial differentiation on ultrastructural analysis. Interestingly, two of these cases (cases 1 and 7) showed no evidence of cytokeratin expression immunohistochemically. Despite these findings, however, we note that in the majority of these tumors, epithelial features are not fully developed. This view is supported by the finding of only abortive epithelial differentiation ultrastructurally in the form of prominent cell junctions (which alone is not conclusive proof of carcinoma) in four cases, and the relatively low percentage of cases that label for cytokeratin compared to RCC. Moreover, even the cytokeratin-positive cases demonstrated only focal labeling of individual cells despite the application of a broad panel of anti-cytokeratin reagents. Hence, we cannot consider these tumors to be typical carcinomas, particularly in light of the above features that also link them to ASPS.

We suspect that the differences between soft tissue ASPS and these renal tumors is related to the structure of the t(X;17)(p11.2;q25). Most chromosome translocations are usually reciprocal, resulting in two opposite fusion genes, one on each of the two derivative chromosomes, with no net gain or loss of genetic material.27 The reciprocal fusion gene is sometimes also transcribed although the encoded reciprocal fusion protein is rarely functionally significant. The t(X;17)(p11.2;q25) of soft tissue ASPS is peculiar insofar as it appears unbalanced in most cases analyzed cytogenetically, an observation now supported by our recent molecular studies in which the reciprocal TFE3-ASPL transcripts were found in only 1 of 13 cases tested1 (including case ASPS-13) and by the current FISH study showing retention of both copies of 17q25.3 telomeric to ASPL in only one of nine ASPS cases (the same case was the exception by both methods). Thus, the ASPL-TFE3 fusion of soft tissue ASPS is associated in almost all cases with allelic loss at 17q25.3 telomeric to ASPL (and gain of Xp sequences telomeric to TFE3) (Figure 14). The additional chromosome X material includes most of the short arm. The possible additional role of these recurrent translocation-associated genomic imbalances in the biology of soft tissue ASPS is unknown.

In contrast, the t(X;17)(p11.2;q25) in the renal tumors described herein was cytogenetically balanced in six of six cases with karyotypic data, including cases 1, 2, and 4 in the present series and three additional published cases (see below and Table 2). We confirmed the balanced nature of the translocation in all seven cases tested by FISH in this series. This was also supported by our demonstration of the reciprocal fusion product, TFE3-ASPL, in five cases. The negative RT-PCR result in case 4 was expected given the cytogenetically identified complex rearrangement that was nonreciprocal (but balanced in terms of 17q25.3 copy number). The negative

### Table 2. Additional Probable Renal ASPL-TFE3 Tumors Previously Reported as Pediatric Renal Cell Carcinomas

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age/sex</th>
<th>Diameter/stage</th>
<th>Immuno-histochemistry</th>
<th>Cytogenetics</th>
<th>Microscopic description in report</th>
</tr>
</thead>
</table>
| Hernandez-Marti et al| 8/M     | 3.5 cm, stage 3 (lymph node) | Not reported          | t(X;17)(p11.2;q25)                    | "Tubular and papillary tumor composed of large clear cells. Their cytoplasm was often foamy."
| Carcao et al         | 6/F     | Unknown, stage 3 (lymph node) | Not reported          | t(X;17)(p11.2;q25) t(1;12)            | "clear cell"                     |
| Heimann et al        | NA      | NA                     | Not reported          | t(X;17)(p11;q25)                      | "clear cell"                     |

NA, not available.

### Table 3. Translocation-Associated Neoplasms Confirmed Molecularly as Primary Renal Tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Translocation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewing sarcoma/peripheral primitive neuroectodermal tumor</td>
<td>t(11;22)(q24;q12)</td>
<td>Quezado et al31, Parham et al32</td>
</tr>
<tr>
<td>Synovial sarcoma (classic embryonal sarcoma of the kidney)</td>
<td>t(X;18)(p11;q11)</td>
<td>Argani et al33</td>
</tr>
<tr>
<td>Infantile fibrosarcoma (cellular congenital mesoblastic nephroma)</td>
<td>t(12;15)(p13;q25)</td>
<td>Rubin et al34, Knezevich et al35</td>
</tr>
<tr>
<td>Clear cell sarcoma of soft parts (malignant melanoma of soft parts)</td>
<td>t(12;22)(q13;q12)</td>
<td>Rubin et al36</td>
</tr>
<tr>
<td>Alveolar rhabdomyosarcoma</td>
<td>t(1;13)(q36;q14) or t(2;13)(q35;q14)</td>
<td>F. Barr, M. K. Fritsch, P. Argani,* unpublished observations</td>
</tr>
<tr>
<td>ASPL-TFE3 tumors related to alveolar soft part sarcoma</td>
<td>t(X;17)(p11.2;q25)</td>
<td>Current report</td>
</tr>
</tbody>
</table>

* PAX-FKHR fusion was not further subtyped in this case.
cases of primary renal cell carcinomas are often nonimmunoreactive for cytokeratin, although an explanation has not been evident. It is interesting that pediatric renal tumors are not associated with any consistent loss or gain of genetic material from the rearranged chromosomes. The finding of distinctive tumors being associated with balanced and unbalanced forms of the same translocation is, to our knowledge, entirely novel. A re-examination of other translocations in light of this unexpected observation may be warranted. Perhaps the differences in gene dosage or the presence of a functional TFE3-ASPL fusion product (in some cases) account for the differing morphology and pattern of spread of these renal tumors. Another possibility is a role for the loss of the only functional copy of TFE3 in these renal tumors (but not in ASPS), given that TFE3 is subject to X-inactivation and assuming that the t(X;17) in females is involving the active X. Alternate hypotheses are that the clinicopathological differences reflect (i.e., a renal tubular cell versus a primitive mesenchymal cell of soft tissue) differences between the renal and soft tissue precursor cells in which the t(X;17) occurs, and/or the local effects of the renal environment on the tumor. In support of the latter hypothesis, other primary renal sarcomas such as clear cell sarcoma of the kidney frequently spread to renal hilar lymph nodes, perhaps because of the rich lymphatic drainage system inherent to the kidney. Also, the consistent calcifications in these tumors may relate to the prominent role the renal parenchyma plays in transporting calcium to preserve it from urinary wastage. Regardless, given the significant genetic and histopathological differences between the ASPL-TFE3 renal tumors and soft tissue ASPS, we consider these neoplasms to be distinct entities.

As previously mentioned, we note three additional renal tumors (aside from case 2 of this series), reported as carcinomas in the literature, that demonstrated a cytogenetically balanced t(X;17)(p11.2;q25) in each case (Table 2). None of these tumors were illustrated nor were any immunohistochemical stains reported. Two of the tumors presented with lymph node metastasis, consistent with the trend in our series. All of these tumors had clear cell morphology. We strongly suspect that these represent additional examples of the presently described entity. Interestingly, it has long been noted that pediatric renal cell carcinomas are often nonimmunoreactive for cytokeratins, although an explanation has not been evident. It is likely that some of those cytokeratin-negative carcinomas are additional examples of these ASPL-TFE3 tumors.

Although the renal tumors we report all occurred in patients younger than 20 years of age, our experience is somewhat biased toward pediatric renal tumors given the nature of the NWTSPC. We note that soft tissue ASPS affects a broader age range than the lesions we have identified in the kidney. Although ASPS is typically a tumor of children and young adults, the 0 to 20 year age range that encompasses our renal tumors accounts for only 37% of ASPS cases. This suggests that additional cases of primary renal ASPL-TFE3 lesions could be found among adult renal tumors, perhaps among the 6 to 7% of unclassifiable carcinomas or among tumors classified as conventional (clear cell) or papillary carcinoma, particularly those that are cytokeratin-negative. However, two lines of evidence suggest that the ASPL-TFE3 renal tumors preferentially affect young patients. First, the renal tumors reviewed at MSKCC were not biased toward young patients; in fact, they were predominantly from adults. The ASPL-TFE3 tumors identified within this group were identified purely on morphological grounds without knowledge of the patients’ ages, and they proved to be from the minority of tumors resected from young patients.

Finally, we again note the predilection of translocations that classically are associated with soft tissue tumors to occur in primary renal tumors (Table 3). With the exception of infantile fibrosarcoma, clear cell sarcoma of soft parts, and these ASPL-TFE3 renal tumors, all of the other tumors were previously usually considered to be Wilms tumor variants before molecular analysis allowed their definitive distinction. Separation of these entities from Wilms tumor has allowed the clinical behavior of the latter to be better understood. Distinction of primary renal ASPL-TFE3 tumors from true pediatric RCCs should help allow us to better understand the clinicopathological features of both, which should lead to more appropriate management approaches.

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