

Renal Cell Carcinoma Associated with Xp11.2 Translocation: Clinicopathologic and Immunohistochemical Findings of 4 Cases

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Background : The new WHO classification includes the recently described renal cell carcinomas (RCC) that are associated with several different translocations, involving chromosome Xp11.2, and they all result in gene fusions involving the TFE3 gene. The authors describe the clinicopathologic and immunohistochemical findings of 4 patients who had the morphologic features of RCC with Xp11.2 translocations. **Methods :** Among 9 surgically resected and pathologically proven pediatric RCCs, 4 showed a typical RCC histopathology with the Xp11.2 translocation. Immunohistochemical stains were performed for TFE3, AE1/AE3, epithelial membrane antigen, vimentin, HMB45, S-100 protein and CD10. **Results :** The 4 study subjects included one male and 3 females, and their chief complaints were gross hematuria and abdominal pain. Histologically, the tumors showed two different histologic types: type 1 tumors (2 cases) that corresponded to those of ASPL-TFE3 RCC, and type 2 tumors (2 cases) that corresponded to PRCC-TFE3 RCC. Nuclear TFE3 immunostaining was seen in 3 cases. All the tumors were immunoreactive for CD10, and vimentin and cytokeratin were expressed in 3 cases and HMB-45 was expressed in 2 cases. **Conclusions :** Our results show that significant numbers of pediatric RCC are translocation-related. Therefore, when one encounters an RCC in the pediatric population, the possibility of a translocation-related RCC should be kept in mind.

Key Words : Renal cell carcinoma; TFE3; Translocation; Child

The new WHO classification of RCCs includes the recently described RCCs that harbor the ASPL-TFE3 gene fusion or the PRCC-TFE3 gene fusion. Collectively, these tumors have been termed Xp11.2 or TFE3 translocation carcinomas. These tumors predominantly occur in children and young adults, and their most distinctive histopathologic appearance is of a papillary architecture that is comprised of clear cells with a frequent nested architecture, and often there are cells having granular eosinophilic cytoplasm. Approximately 44 cases of renal carcinomas associated with Xp11.2 translocations have been reported in the literature.^{1,2} However, there had been no published report on this tumor in Korea, though one case was presented and discussed at a monthly slide conference of the Korean Society of Pathologists in 2003.

With the advent of the new 2004 WHO RCC classification, the authors reviewed 9 cases of pediatric RCC, and 4 of which were found to have the histopathologic findings of renal carcinomas associated with Xp11.2 translocations. We describe here the clinicopathologic and immunohistochemical findings of these 4 RCC cases with Xp11.2 translocations.

MATERIALS AND METHODS

The authors collected the medical records and specimens of 9 cases of RCC that were diagnosed at a patient age younger than 18 years, among 193 cases of RCC from the Department of Pathology of the Samsung Medical Center during a 10-year period from 1995 to 2004. All the glass slides of these 9 children with RCC were reviewed. The original pathologic diagnosis of these 9 cases was papillary RCC in 4 patients, oncocytic RCC in one patient and conventional RCC in 4 patients. Of these 9 cases, 4 showed the similar histopathologic findings of renal carcinomas associated with Xp11.2 translocations. The clinical and pathologic findings of all 4 patients with RCC associated with Xp11.2 translocations were reviewed. Gross photographs of all cases, except the needle biopsy of case 4, were available for review. All the specimens were fixed in 10% neutral formalin, and hematoxylin-eosin staining was performed after routine histological processing. Immunohistochemistry was carried using the avidin-biotin complex method on the representative paraffin-embedded tumor tissue sections. The primary antibodies included cytotok-

eratin (AE1/AE3, 1:80, Zymed, South San Francisco, California, USA), epithelial membrane antigen (EMA; 1:200, DAKO, Clostrup, Denmark), vimentin (1:1,000, DAKO, Clostrup, Denmark), CD10 (1:50, Novocastra, Newcastle upon Tyne, UK), S-100 protein (1:2,000, DAKO, Clostrup, Denmark), TFE3 (1:500, Santa Cruz, Biotechnology, Santa Cruz, CA, USA) and HMB-45 (1:40, DAKO, Clostrup, Denmark).

RESULTS

The clinical and pathologic findings of the 4 patients are summarized in Table 1 and 2. The patients' ages ranged from 8 to 15 years with a mean age of 12.7 years. The ratio of males and females was 1:3. All the tumors were unilateral tumors. Three patients underwent radical nephrectomy for renal masses. One patient (case 4) had been diagnosed by needle biopsy at 15 years of age because of advanced stage IV disease with mediastinal metastasis. Three patients were in stage I (cases 1, 2 and 3) and 1 patient was in stage IV (case 4). Three patients are still alive (cases 1, 2 and 3) and one patient (case 4) was lost after a follow-up of 5 months. Two patients presented with gross hematuria (cases 1 and 2) and one of the patients presented with gross hematuria of one year's duration (case 1). One patient presented with

abdominal pain (case 4) and one patient was incidentally found to have a renal mass (case 3).

Grossly, all tumors were well-circumscribed, tan brown or yellow with or without hemorrhagic or necrotic foci. The mean tumor diameter was 6 cm, and this ranged from 2 cm to 15 cm. The tumors were bulging out with a papillary appearance on the cut sections (Fig. 1). Renal masses were discovered at the lower pole of the right kidney (case 1), at the upper pole of the left kidney (case 2), at the mid-area of the left kidney (case 3), respectively. One patient presented with a huge mass in the right kidney with mediastinal metastasis (case 4).

The tumors were classified into two different types according to their histology. Cases 1 and 2 were categorized as type 1, and cases 3 and 4 were classified as type 2. Type 1 tumors showed a papillary architecture comprised of voluminous clear to eosinophilic cells, and there were vascular cores with hyalinized nodules and psammoma bodies. The tumor cells were polygonal or tall columnar, and their nuclei were round and vesicular with frequent nuclear grooves or there was a single prominent nucleoli (Fig. 2A). Small satellite tumors were present around the main masses. Lymphocytes and plasma cells had infiltrated into the fibrovascular cores of the papillary structures within the tumors. On the other hand, the type 2 tumors were composed of compact nests of round to polygonal cells with abundant clear or eosinophilic cytoplasm (Fig. 3A). The type 2 tumor cells were smaller than those of type 1 tumors, but their nuclear features were similar. Infiltrates of inflammatory cells were seen in the fibrous stroma within one tumor (case 3). Some renal tubules and glomeruli were entrapped at the tumor periphery in case 3. Some of the entrapped tubules showed immature metaplasia. Characteristically, all the tumors showed considerable amounts of small, round to rod shaped, granular bodies in abundant clear or eosinophilic cytoplasm.

Table 1. Clinical summary of 4 patients with Xp11.2 translocated RCCs

Cases	Sex	Age (year)	Stage	Treatment	Follow up
1	F	15	I	Radical nephrectomy	NED, 70 months
2	F	8	I	Radical nephrectomy	NED, 16 months
3	M	13	I	Radical nephrectomy	NED, 24 months
4	F	15	IV	Needle biopsy	LOF, 5 months

NED, no evidence of disease; LOF, loss of follow-up.

Table 2. Summary of histopathologic features of 4 patients with Xp11.2 translocated RCCs

Cases	Pattern	Nuclei	Cytoplasm	Cell border	Other findings
1	Papillary and nested	Vesicular Irregular Single nucleoli	Clear to eosinophilic Granular Rod-shape bodies	Distinct	Hyalinized fibrovascular cores with psammoma bodies Satellite nodules
2	Papillary and nested	Vesicular Irregular Single nucleoli	Clear to eosinophilic Granular Rod-shape bodies	Distinct	Hyalinized fibrovascular cores with psammoma bodies Satellite nodules Extracapsular tumor invasion
3	Compact architecture Nested Focally papillary	Smaller Single nucleoli	Clear to eosinophilic	Distinct	Fibrous stroma with inflammatory cells Entrapped immature tubules and embryonal metaplasia Calcification adjacent renal parenchyma
4	Compact architecture Nested Focally papillary	Smaller Single nucleoli	Clear to eosinophilic	Distinct	

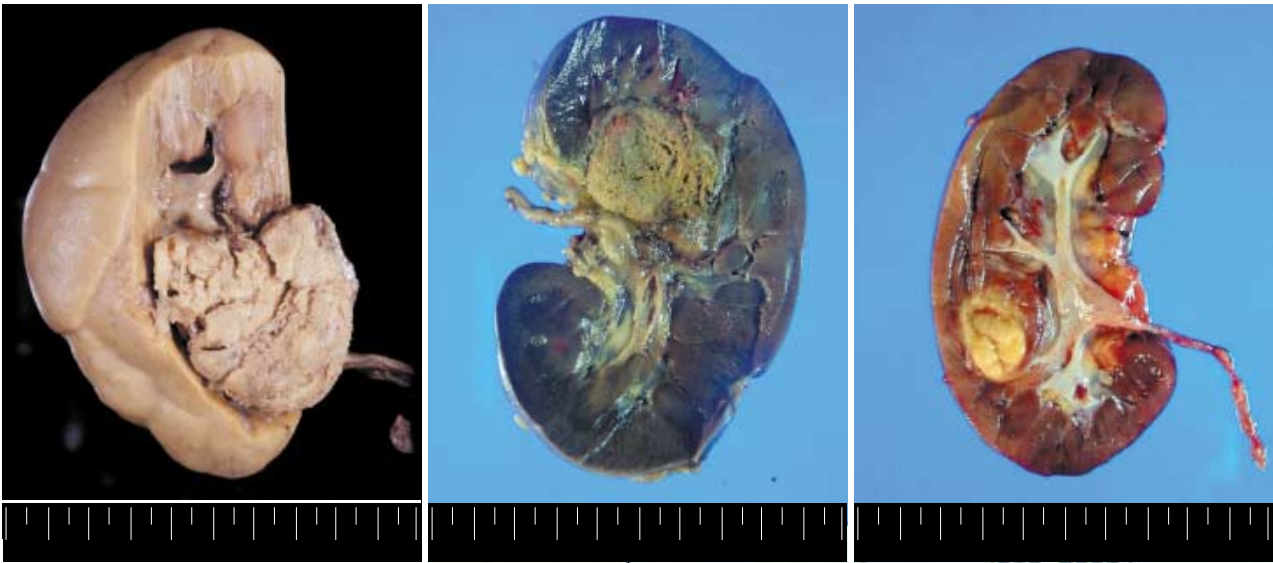


Fig. 1. Cut surfaces show well-circumscribed, yellow to tan brown masses (cases 1, 2 and 3, respectively).

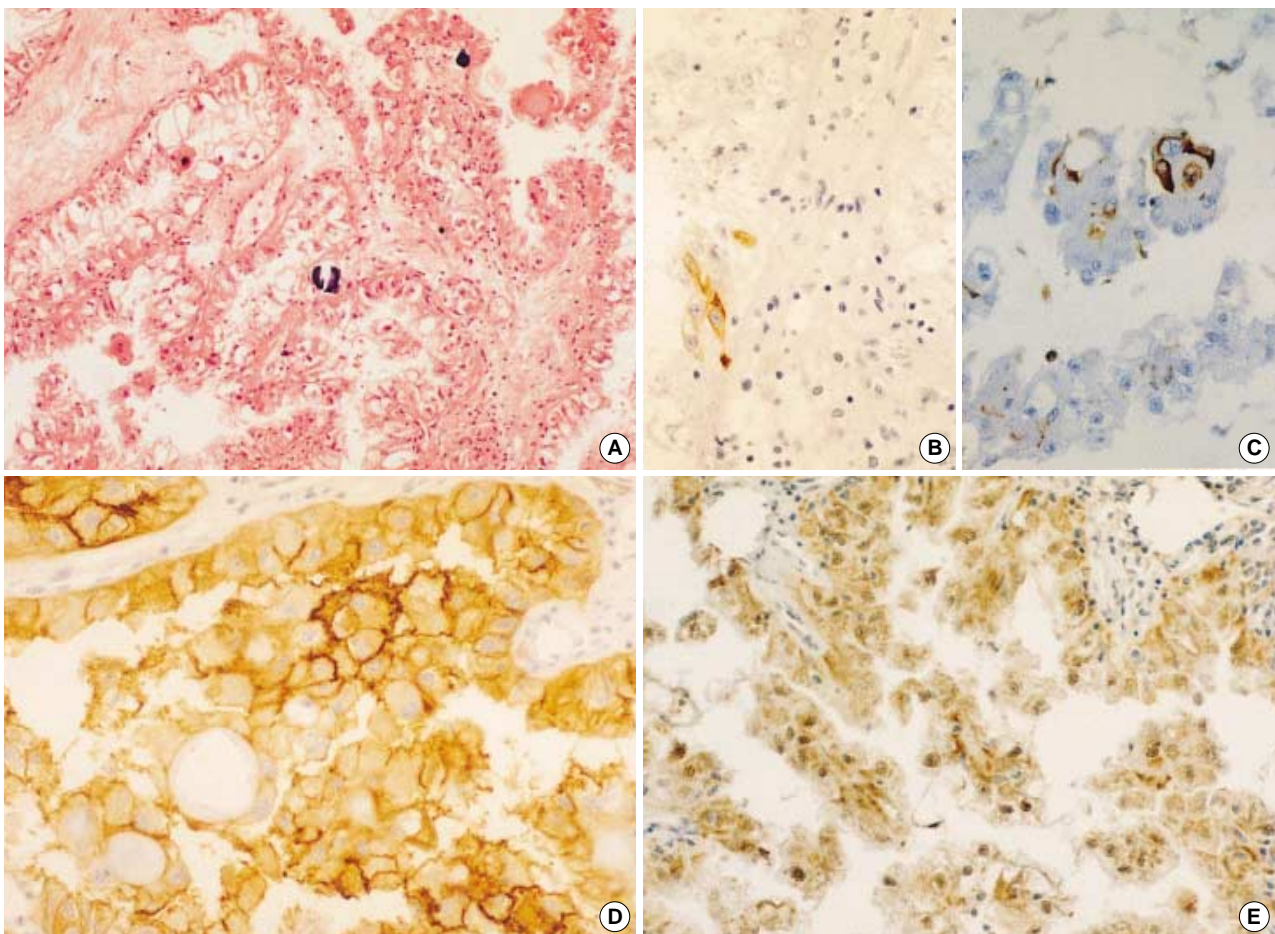


Fig. 2. (Case1 and 2) (A) The tumor shows papillary, voluminous clear to eosinophilic cells and vascular cores with psammoma bodies (type 1). (B&C) Tumor cells show focal positivity for cytokeratin (B) and vimentin (C). (D) CD10 shows diffuse immunoreactivity. (E) Tumor cells show nuclear and cytoplasmic immunoreactivity for *TFE3*.

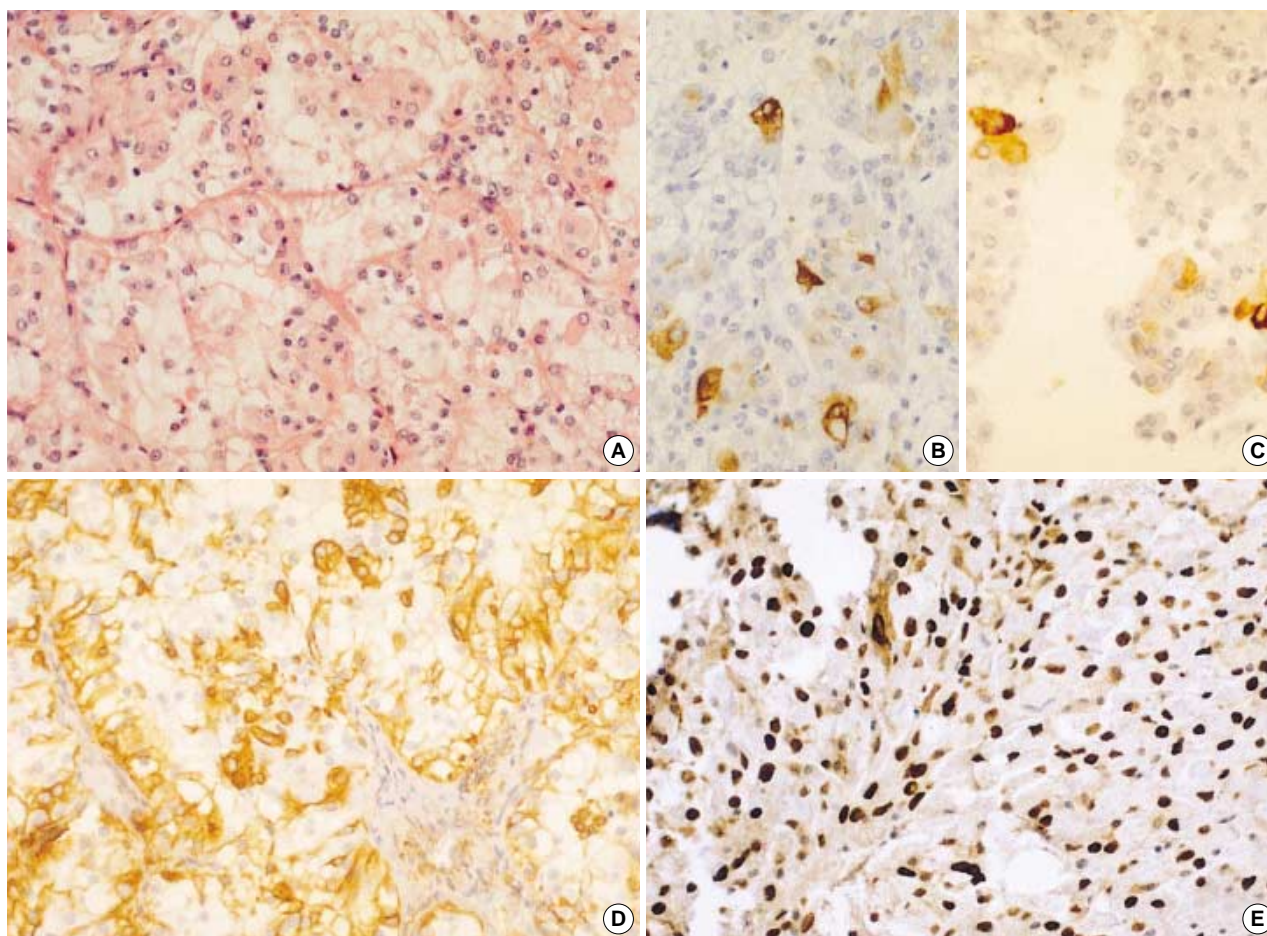


Fig. 3. (Case 3 and 4) (A) The tumor shows compact pattern with abundant clear to eosinophilic cytoplasm. (type 2) (B&C) Tumor cells show focal positivity for HMB-45 (B) and CD10 (C). (D) Vimentin shows focal immunoreactivity. (E) Tumor cells show *TFE3* nuclear immunoreactivity.

Table 3. Immunohistochemical findings of 4 patients with Xp11.2 translocated RCCs

case	CK (AE1/AE3)	CD10	Vimentin	EMA	S-100	HMB-45	TFE3
1	focal positive	positive	focal positive	negative	negative	negative	nuclear and cytoplasmic
2	rarely positive	positive	negative	negative	negative	negative	nuclear and cytoplasmic
3	negative	focal positive	focal positive	negative	negative	positive	cytoplasmic
4	negative	focal positive	negative	negative	negative	positive	nuclear

CK, cytokeratin; EMA, epithelial membrane antigen; TFE3, candidate 1-transcription factor binding to enhancer 3.

The immunohistochemical findings are summarized in Table 3. Nuclear immunoreactivity for TFE3 was observed in 3 cases (cases 1 and 2, Fig. 2E and case 4, Fig. 3E). All the tumors showed CD10 immunoreactivity with a diffuse pattern in the type 1 tumors (Fig. 2D) and a focal pattern was seen in type 2 tumors (Fig. 3C, D). Vimentin was focally expressed in cases 1 and 3 (Fig. 2C). AE1/AE3 was focally positive in case 1 (Fig. 2B), but AE1/AE3 was rarely positive in a few tumor cells of case 2. Case 3 and 4 were negative for AE1/AE3. HMB-45 staining was only observed in type 2 tumors (Fig. 3B); this staining was diffuse and

strong in case 3 and it was localized to 20% of the tumor cells in case 4. EMA and S-100 protein were not expressed in any cases.

DISCUSSION

RCC in children and young adults is a rare finding. A total of 193 surgically resected and pathologically proven RCCs were found in the Department of Pathology at the Samsung Medical Center for the past 10 years. There were 9 patients (4.7%) younger

than 18 years. Of the 9 pediatric RCCs, 4 (44.4%) were found to have the histopathologic findings of Xp11.2 translocation carcinoma. When considering that fewer than 4% of renal tumors in children and young adults are RCCs, it is evident that the Xp11.2 translocation carcinomas seen in this series comprised a significant percentage of the pediatric RCCs.

Xp11.2 translocation carcinoma predominantly affects children and young adults, though a few older patients have been reported on.² The patients' ages have ranged from 1 to 77 years with a mean of 23.5 years.^{1,2} There is no definite gender predominance. The clinical manifestations of these tumors include hematuria, urinary tract infection, abdominal mass and flank pain. Almost all patients have associated lymph node metastasis at the time of diagnosis.³ The most distinctive histopathologic appearance is a papillary carcinoma composed of clear cells, but these tumors frequently have a more nested architecture and cells having granular eosinophilic cytoplasm. Papillary RCCs also show a papillary architecture, but the tumor papillae contain a delicate fibrovascular core; aggregates of foamy macrophages and cholesterol crystals. Occasionally the papillary cores are expanded by edema or hyalinized connective tissue and the tumor cells have less voluminous scanty cytoplasm. The morphologic appearances of carcinomas that are associated with specific chromosome translocation breakpoints are variable. The alveolar soft part sarcoma-transcription factor binding to IGHM enhancer 3 (ASPL-TFE3) renal carcinomas are characterized by cells having voluminous, clear to eosinophilic cytoplasm, discrete cell borders, vesicular nuclear chromatin and prominent nucleoli.³ In contrast, the papillary RCC-transcription factor binding to IGHM enhancer 3 (PRCC-TFE3) renal carcinomas generally show less abundant cytoplasm, fewer psammoma bodies, fewer hyaline nodules and a more nested, compact architecture.³ The PRCC-TFE3 RCCs may have a less invasive growth pattern than the ASPL-TFE3 RCCs.⁴ Vascular invasion and the tendency for nodal involvement have been frequently identified in ASPL-TFE3 RCCs.⁴ It is likely that the PRCC-TFE3 RCCs are slow-growing indolent lesions and this assertion is supported by the identified low mitotic rate, the extremely low KI-67 index, and the high frequency with which a calcified fibrous pseudocapsule is seen around these tumors.⁴ Ultrastructurally, PRCC-TFE3 renal carcinomas most closely resemble conventional RCCs in that they feature cell junctions, microvilli, intracytoplasmic fat and glycogen, whereas most of the ASPL-TFE3 renal carcinomas demonstrate membrane-bound cytoplasmic granules and a few membrane-bound rhomboidal crystals that are identical to those seen in alveolar soft-part sarcoma (ASPS) of the soft tis-

sue. PRCC-TFE3 renal carcinomas have occasionally demonstrated distinctive intracisternal microtubules that are similar to those seen in malignant melanoma and extraskeletal myxoid chondrosarcoma. The immunoprofile of Xp11.2 translocation carcinoma has been reported to differ from that of conventional RCC. A majority of conventional RCCs react with such epithelial markers as cytokeratin and EMA, but these epithelial markers are expressed in only about 50% of the renal carcinomas with Xp11.2 translocation.^{1,2} The CD 10 expression was investigated in primary (n=180) and metastatic (n=58) RCCs, and 154 (90%) of the 172 primary RCCs and 48 (86%) of the 56 metastatic RCCs were found to express CD10.⁵ The most distinctive immunohistochemical feature of renal carcinoma with a Xp11.2 translocation is the nuclear labeling for TFE3. The TFE3 gene is a member of the basic-helix-loop-helix family of transcription factors. The unbalanced translocation results in the fusion of the TFE3 gene into a novel gene named ASPL on chromosome 17q25 or PRCC on chromosome 1q21.2.

Our cases showed two different histological types. Type 1 tumor was easily distinguished from the conventional or papillary RCCs by its predominant papillary architecture; this was composed of distinctly prominent voluminous clear cells and psammoma bodies. Type 1 tumors correspond histologically to ASPL-TFE3 renal carcinomas, as described by Argani *et al.*³ and Renshaw *et al.*⁶ Type 2 tumors are characterized by a more solid or compact architecture, a less prominent papillary architecture and slightly less voluminous cells, which correspond to renal carcinomas with the PRCC-TFE3 phenotype. It is interesting that all 4 tumors showed ASPS-like fine granular round or rod shaped cytoplasmic bodies. In the present cases, the observed immunoreactivities were similar to those previously reported, except for the HMB-45 reactivity seen in two cases. CD10 expression was consistently observed, and TFE3 nuclear staining was observed in 3 tumors. None of our cases showed EMA or S-100 protein expression. Both vimentin and cytokeratin were focally expressed in 2 cases of type 1 and 2 each, and HMB-45 was only expressed in the two type 2 cases. As might be expected for a transcription factor, normal TFE3 is located in the nucleus, and nuclear TFE3 immunoreactivity was suggested to be highly sensitive and specific.⁷ The present study shows cytoplasmic and nuclear staining in 2 cases, strong nuclear staining in a single case and strong cytoplasmic staining in a single case. The remaining 5 pediatric patients with conventional and papillary RCCs revealed negative nuclear and cytoplasmic immunoreactivity for TFE3. The cytoplasmic expression of TFE3 has also been described in a couple of immunohistochemistry based investi-

gations^{3,8}, and this was ignored by Argani and coworkers because native TFE3 and its fusion proteins are known to be localized to the nucleus.⁷ However, Argani and coworkers also suggested, because of technical reasons, that a negative result for TFE3 may not entirely exclude a diagnosis of translocation renal carcinoma.⁷ One case in this study (case 3) showed only cytoplasmic immunoreactivity for TFE3. However, it was included in this study because its histologic features were similar to the translocation RCC and the immunoprofiles, including HMB-45 positivity, were different from the conventional RCC. Bruder *et al.*¹ reported that the nuclear TFE3 overexpression was negative, in two of the eight carcinomas with voluminous cytoplasm. Two of our cases showed immunoreactivity for HMB-45, and negativity for the epithelial markers. Immunoreactivity for HMB-45 in RCC has been described in renal carcinomas with t(6;11)(p21;q12), and histologically, these carcinomas are predominantly composed of nests of polygonal cells with well-defined cell borders that are delineated by thin capillaries. In addition, 10 to 50 cell clusters of smaller cells characteristically surrounded small round nodules of hyaline, basement membrane-like material, and this yielded an appearance reminiscent of Call-Exner bodies.⁹ Our two tumors differed histologically from the renal carcinomas with t(6;11)(p21;q12). Although molecular analysis was not performed in this study, the histologic and immunohistochemical features of our cases including a case with no TFE3 nuclear staining are compatible with the renal carcinoma with the Xp11.2 translocation.

The first reported translocation in RCC was t(X;1)(p11.2;q21), which results in the fusion of the PRCC and TFE3 genes.^{10,11} Several different translocations involving chromosome Xp11.2 have been reported and they include t(X;1)(p11.2;q21), t(x;1)(p11.2;p34), inv(x)(p11;q12), t(6;11)(p21;q12) and t(x;17)(p11.2;q25).

In conclusion, the renal carcinomas associated with Xp11.2 translocations/TFE3 gene fusion demonstrated characteristic histologic and immunohistochemical features that are distinguishable from those features seen in the conventional RCCs. Because translocation renal carcinomas comprise a significant proportion of pediatric renal carcinomas, they should be included in the differential diagnosis of pediatric renal neoplasms.

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