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Case Report

Xp11.2/*TFE3* translocation-associated renal cell carcinoma with multilocular cystic structures in an elderly patient: a case report

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Abstract: Background: Xp11.2 translocation-associated renal cell carcinomas (Xp11.2-RCCs) are a rare subtype of renal neoplasm. The incidence of these tumors is lower in the elderly than in children and young adults. Furthermore, there have been very few reports of Xp11.2-RCCs with multilocular cystic structures. Case: A 66-year-old Japanese female was admitted to our hospital with amultilocular cystic lesion in her right kidney. Computed tomography revealed that the lesion occupied the cortex and medulla of the upper pole of the right kidney, and partial nephrectomy was performed. Resected tissue contained a multilocular cystic tumor measuring 4.5×4.3×4.0 cm. Histologically, the cyst walls were composed of thin fibrous septa covered by a few layers of tumor cells with a clear cytoplasm. Psammoma bodies were scattered in the cyst lining. Immunohistochemically, most of tumor cells were positive for TFE3, α-methylacyl CoA racemase (AMCAR), and cathepsin K. Tumor cells also showed immunoreactivities for CD10 and cytokeratins, but were negative for carbonic anhydrase 9 and c-kit. Fluorescence *in situ* hybridization using a break-apart probe revealed monoalleleic *TFE3* breakage. Based on these results, the tumor was diagnosed as Xp11.2-RCC, even though the patient's age and morphological architecture were not representative. Conclusion: This case demonstrated that Xp11.2-RCCs may occur in adults with multilocular cystic structures. Retrospectively, the recognition of psammoma bodies represented an important diagnostic sign.

Keywords: Xp11.2/*TFE3* translocation-associated renal cell carcinoma (Xp11.2-RCC), elderly, multilocular cystic structures, *TFE3*, fluorescence *in situ* hybridization (FISH)

Introduction

The pathological subtypes of renal cell carcinomas (RCCs) have greatly expanded with the accumulation of cytogenetic information due to technological improvements in pathology laboratories. Xp11.2/*TFE3* translocation-associated renal cell carcinomas (Xp11.2-RCCs) are characterized by chromosomal translocations involving the *TFE3* (*transcription factor enhancer 3*) gene located at chromosome Xp11.2. *ASPL*, *PRCC*, *NONO*, *PSF*, and *CLTC* have been identified as fusion partners paired with the *TFE3* gene [1, 2]. Furthermore, a novel fusion gene was recently reported [3]. Regardless of the fusion partner, these translocations typically induce the excessive nuclear accumulation of the TFE3 protein in Xp11.2-RCC, which is detected by immunohistochemical staining of the TFE3 protein. Although Xp11.2-RCCs constitute 20-70% of pediatric RCCs, the incidence of adult cases is markedly lower (1.6-4.2%) [4]. A previous study reported that Xp11.2-RCCs accounted for less than 1% of adult RCCs [5]. However, the actual incidence of this disease is larger in adults than in children.

Clinically, Xp11.2-RCCs are mostly diagnosed at an advanced stage with metastatic disease. Although pediatric cases are generally con-

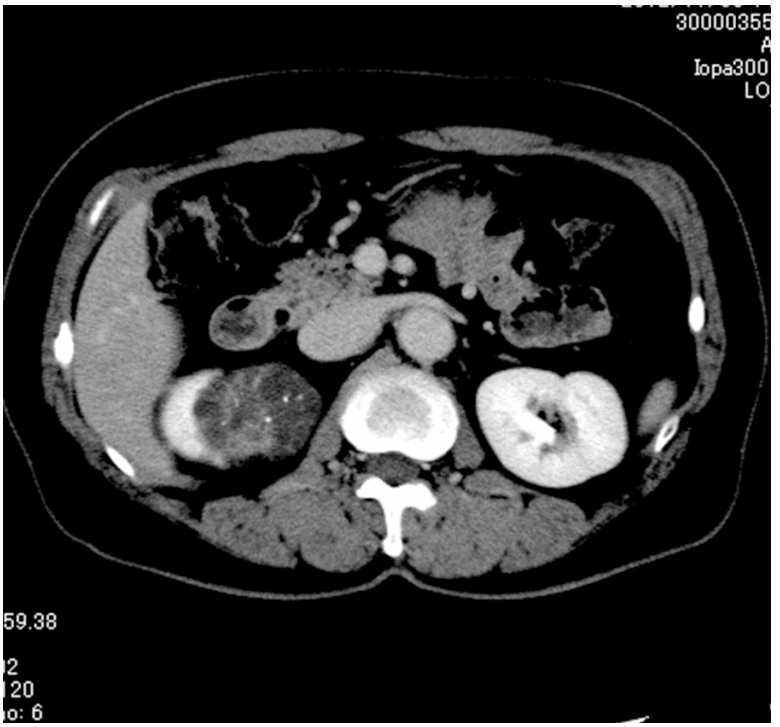


Figure 1. Preoperative enhanced computed tomography image. A multilocular cystic mass with minute calcifications, expanding from the pelvis to the upper pole of the fight kidney, measuring 5.5×5.3×4.5 cm in size.

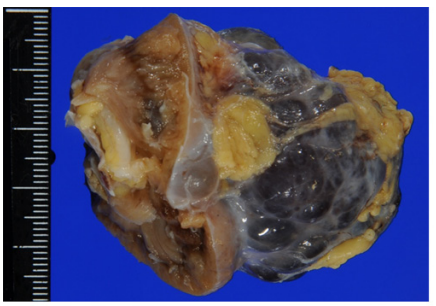
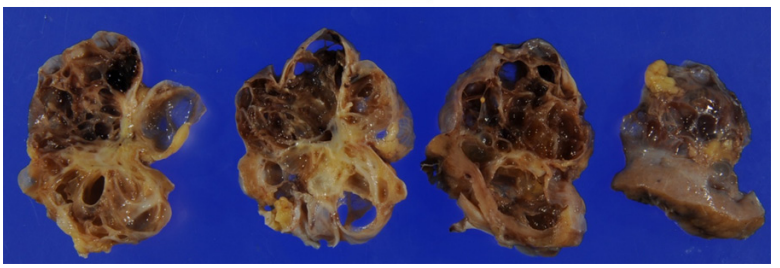


Figure 2. Macroscopic findings of the tumor. The tumor was brown or gray in color and measured 5.5×5.0×4.8 cm. The cut section of the tumor showed a multilocular cystic lesion with a focally fibrotic area. The walls of the cysts were uniformly thin and white to brown in color. Minute calcifications were scattered.



trolled successfully, adult cases are more aggressive. There is currently no specialized therapeutic strategy for advanced Xp11.2-RCCs. A precise diagnosis of Xp11.2-RCC is considered important for establishing an effective therapeutic regimen other than surgical resection. However, a pathological diagnosis of adult Xp11.2-RCC is challenging, because clear cell RCCs occupy the overwhelming number as differential diagnostic candidates.

Although information regarding gross findings is limited, most Xp11.2-RCCs form well-demarcated solid tumors, while only a few form cystic lesions. Histologically, Xp11.2-RCCs with specific fusion partners have been reported to display some distinctive features: *ASPS-TFE3*-associated tumors are more likely to have a voluminous clear cytoplasm, discrete cell borders, and an alveolar or papillary growth pattern with psammoma bodies, where as *PRCC-TFE3*-associated tumors have a nested growth pattern, smaller cells with a less abundant cytoplasm, and fewer calcifications [6]. Nevertheless, a differential diagnosis from clear cell and papillary RCCs is challenging during routine diagnostics.

We herein presented an unusual case of Xp11.2-RCC occurring in an elderly patient with a multilocular cystic architecture. We successfully confirmed a chromosomal translocation involving *TFE3* by break-apart FISH using formalin-fixed paraffin-embedded tumor tissue samples. The indication for a precise diagnosis in this case was the existence of psammoma bodies scattered in the tumor.

Case report

A 66-year-old Japanese female had a cystic lesion in her right kidney for 30 years without any subjective symptoms. Her family and previous histories were not contributory. A computed tomography scan revealed a multilocular cystic mass with minute calcifications, expanding from the pelvis to the upper pole of the right kidney, measuring 4.5×4.3×4.0 cm in size

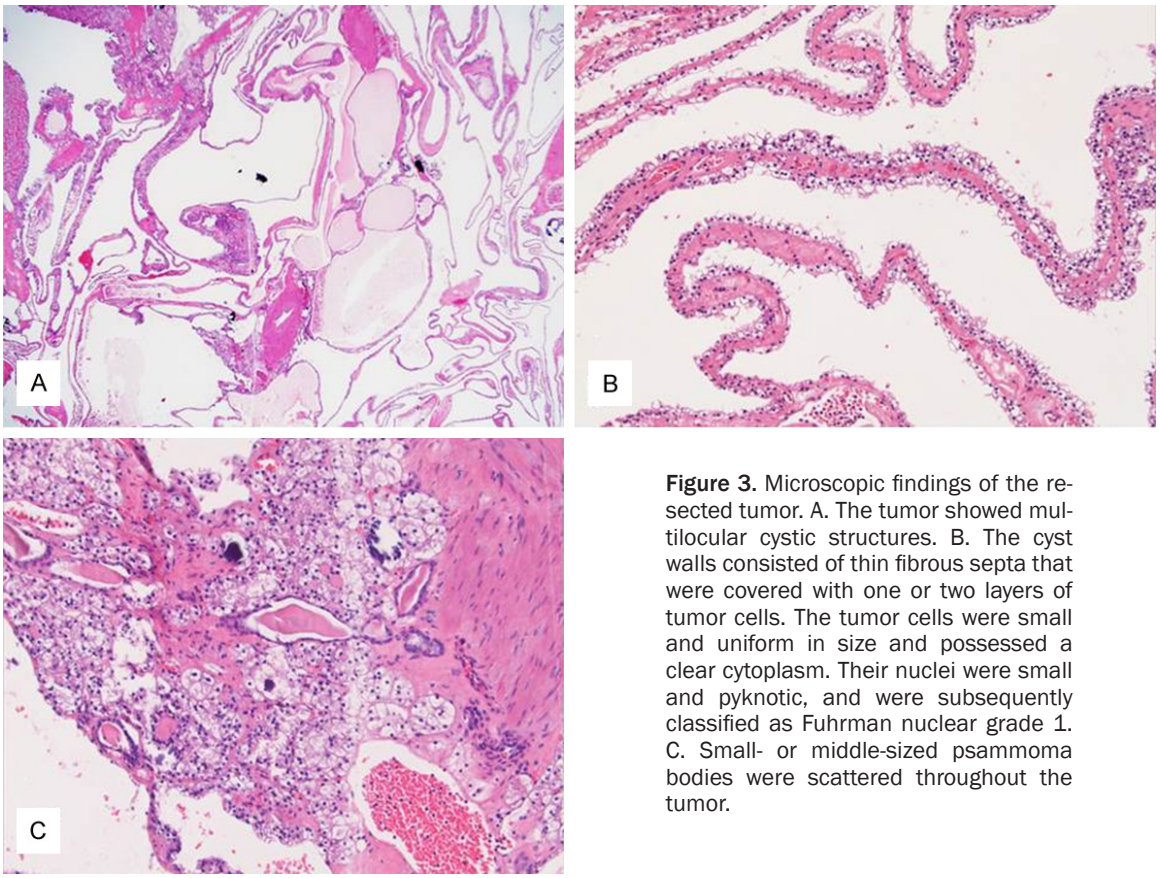


Figure 3. Microscopic findings of the resected tumor. A. The tumor showed multilocular cystic structures. B. The cyst walls consisted of thin fibrous septa that were covered with one or two layers of tumor cells. The tumor cells were small and uniform in size and possessed a clear cytoplasm. Their nuclei were small and pyknotic, and were subsequently classified as Fuhrman nuclear grade 1. C. Small- or middle-sized psammoma bodies were scattered throughout the tumor.

Table 1. Results of immunohistochemistry

Antibodies	Source	Clone	Dilution/antigen retrieval	Result
TFE3	Cell Maque	Polyclonal	1:200/TEpH9.0 autoclave 15 min	Positive
Cathepsin K	Abcam	3F9	1:200/TEpH9.0 autoclave 15 min	Positive
Pancytokeratin	Roche	AE1/AE3	-/-	Positive
Cytokeratin 7	DAKO	OV/TL12/30	1:100/Heat (ER1/20 min)	Negative
EMA	Nichirei	E29	-/Heat (ER1/20 min)	Negative
AMACR	Japan Tanner	P504s	1:300/Heat (ER2/20 min)	Positive
CD10	Leica	56c6	-/Heat (ER2/20 min)	Positive
Vimentin	DAKO	V9	1:100/Heat (ER1/10 min)	Positive
PAX8	Abcam	PAX8R1	1:20/Heat (ER2/20 min)	Positive
RCC marker	Leica	66.4.C2	1:20/-	Positive
Melanosome	DAKO	HMB45	1:100/Heat (ER1/10 min)	Negative
CA-IX	Cell Signaling	D47G3	1:200/TEpH9.0 autoclave 15 min	Negative

(**Figure 1**). Since the size of the lesion had gradually increased over the past 6 years, partial nephrectomy was performed.

Pathological findings

Grossly, the resected kidney contained a multilocular cystic lesion measuring 5.5×5.0×4.8 cm. The cyst walls were uniformly thin, and white to brown in color. The lesion had a well-demarcated margin and compressed the neighboring normal renal parenchyma (**Figure 2**).

A microscopic examination of hematoxylin and eosin-stained sections revealed a multilocular cystic tumor (**Figure 3A**). The cyst walls consist-

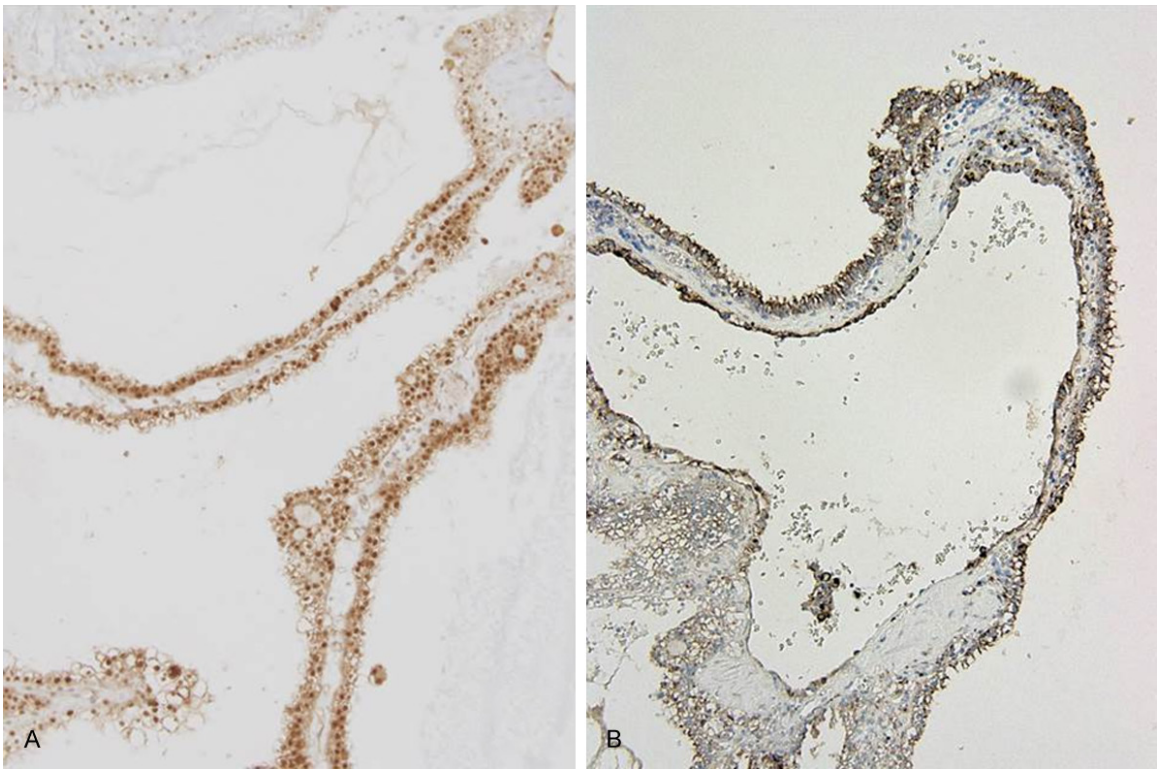


Figure 4. Immunohistochemical features of the resected tumor. A. Most tumor cells were positive for TFE3. B. Tumor cells were diffusely positive for cathepsin K.

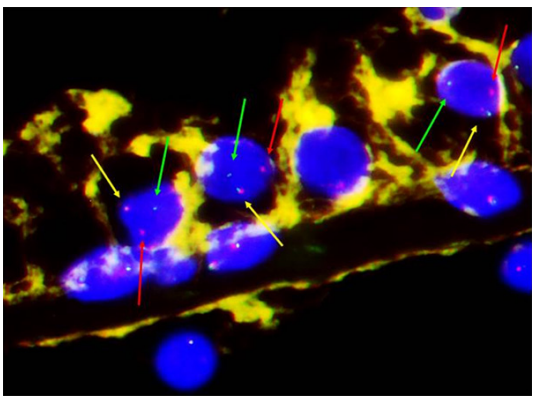


Figure 5. TFE3 FISH image. A pair of fused signals (yellow arrows) and a pair of separated signals (red-green arrows) were observed in many of the nuclei in tumor cells, showing the typical patterns of TFE3 rearrangements.

ed of thin fibrous septa that were covered with one or two layers of tumor cells (**Figure 3B**). Focally, a sheet-like growth pattern was also observed without obvious nested structures. The tumor cells were small and uniform in size and possessed a clear cytoplasm. Their nuclei were small and pyknotic, and were subsequently classified as Fuhrman nuclear grade 1. There were few mitotic figures. Psammoma bodies were occasionally detected (**Figure 3C**). Melanin pigmentation was absent.

The results of immunohistochemistry are summarized in **Table 1**. Most tumor cells were positive for TFE3 (**Figure 4A**), α-methylacyl CoA racemase (AMCAR), and cathepsin K (**Figure 4B**). Tumor cells also showed immunoreactivities for CD10 and cytokeratins, but were negative for carbonic anhydrase 9 and c-kit.

In order to confirm the rearrangement of *TFE3*, a fluorescence *in situ* hybridization (FISH) analysis was performed using a Histology FISH Accessory Kit (DAKO, Glostrup, Denmark) and dual-color *TFE3* break-apart probe (GSP Laboratory, Kawasaki, Japan) according to the manufacturer's instructions. In brief, 4-μm-thick formalin-fixed and paraffin-embedded sections were deparaffinized in xylene and rehydrated in graded ethanol. After boiling, specimens were digested with Pepsin, ready-to-use (DAKO) at 37°C for 8 minutes. The dual-color *TFE3* break-apart probe labeled by FITC and Texas-Red was

then applied and incubated at 37°C overnight. After rinsing, the labeled specimen was observed with a fluorescence microscope with a 100× oil immersion objective. In each nucleus, FITC and Texas-Red signals in less than 3 signal diameters were defined as a fused signal. One pair of split signals and one fused signal were detected in 34 out of 100 nuclei (34%) (**Figure 5**). These results demonstrated *TFE3* rearrangements, indicating translocation. Reverse transcription PCR (RT-PCT) to clarify the manner of rearrangement was not successful because the quality of RNA extracted from the FFPE tumor tissue was poor.

Based on these results, the tumor was diagnosed as Xp11.2-RCC. The patient has been doing well without recurrence 2 years after surgery.

Discussion

Xp11.2-RCCs are a newly introduced subtype of RCC. This tumor is characterized by chromosomal translocation involving the *TFE3* gene located at chromosome Xp11.2. The encoding protein, TFE3 belongs to the microphthalmia transcription factor (MiTF)/TFE transcription factor family along with MiTF, TFEB, and TFEC. 6p21/*TFEB* translocation-associated RCCs (6p21-RCCs) have recently been identified. Xp11.2- and 6p21- RCCs occasionally present with melanin pigmentation and positivity for melanocytic markers instead of epithelial markers. Together with the close relationship between TFEs and the melanocyte-differentiation-relating transcription factor, MiTF, perivascular epithelioid cell-derived tumors (PEComas) and translocation-associated RCCs may share some characteristics. Based on these findings, translocation-associated RCCs are now being recognized as MiTF-translocation-associated RCCs.

We herein presented a case of Xp11.2-RCC, which was unique in its multilocular cystic architecture and occurrence in an elderly patient. This tumor was composed of multilocular cysts lined with clear tumor cells of a low nuclear grade. Only five childhood and young adult cases of Xp11.2-RCCs showing multilocular cystic structures have been reported to date [2, 6, 7]. Although the accurate age of tumor onset is unclear for a long-standing follow-up period, this is the first elderly adult case of Xp11.2-RCC showing multilocular cystic structures.

Due to the existence of psammoma bodies, the possibility of Xp11.2-RCC was evaluated by immunohistochemistry followed by break-apart FISH. Differential diagnostic candidates included multilocular cystic RCC and papillary RCC, with the former being a subtype of clear cell RCC. Since tumor cells were low grade and the cytoplasm was watery clear, it was difficult to distinguish multilocular cystic RCC from Xp11.2-RCC. However, the existence of psammoma bodies provided important evidence in a routine HE specimen, which prompted us to perform further immunohistochemical examinations. Immunohistochemically, a negative reaction for carbonic anhydrase 9 led to the diagnosis of multilocular cystic RCC being excluded. Although tubulocystic carcinoma was another diagnostic candidate, it is composed of high grade epithelia with an eosinophilic cytoplasm and thick fibrous septa [8]. Immunohistochemically, similar features to those of papillary RCC were noted, namely, positivity for AMACR. Therefore, tubulocystic carcinoma was also unlikely. We were able to reach a final diagnosis of Xp11.2-RCC based on immunohistochemical positivity for nuclear TFE3 and rearrangement of the *TFE3* gene by break-apart FISH.

The present case provided two important results; (1) Xp11.2-RCCs may occur in the elderly with multilocular cystic structures, and (2) psammoma bodies are an important indication for diagnosing Xp11.2-RCCs and distinguishing then from multilocular cystic RCCs, the closest potential differential candidate.

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Disclosure of conflict of interest

None.

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