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Case Report

Renal cell carcinomas with t(6;11) translocation: identification of a novel Alpha-TFEB fusion point

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Abstract: T(6;11) renal cell carcinoma is a rare tumor with specific chromosomal translocation involving transcription factor EB, which results in an *Alpha-TFEB* gene fusion. It is described as a subtype of the MiT family translocation RCC in the 2016 WHO classification of tumors of the urinary system and male genital organs. Approximately 60 cases were reported, 14 cases of which were confirmed the accuracy fusion points by RT-PCR analysis and sequencing in the literature. Here we reported two new cases with typical morphological characteristics and immunophenotype. FISH analysis confirmed the translocation involving the *TFEB* gene and the diagnosis of t(6;11) renal cell carcinoma in both cases. More importantly, we identified a novel fusion point between *Alpha* nucleotide 1981 and *TFEB* nucleotide 208 upstream of exon3 by RT-PCR and product sequencing in case 1.

Keywords: t(6;11) renal cell carcinomas, Alpha-TFEB, gene fusion, FISH, RT-PCR

Introduction

Renal cell carcinoma (RCC) with t(6;11)(p21,q12) is a rare tumor characterized by a specific chromosomal translocation involving transcription factor EB (*TFEB*). It was proposed as a new entity by the International Society of Urological Pathology (ISUP) Vancouver consensus conference on renal neoplasia [1] and listed in the 2016 WHO classification of tumors of the urinary system and male genital organs.

T(6;11) RCC was firstly described in pediatric patients by Argani in 2001 [2]. In 2003, Davis *et al* [3] and Kuiper *et al* [4] showed that the t(6,11)(p21;q12) results in fusion of the 5' portion of the *Alpha* gene with the transcription factor gene *TFEB* at 6p21, this rusulted in overexpression of TFEB protein. As one of microphthalmia transcription factor (MITF) subfamily, TFEB and another three closely related members (TFE3, TFEC and MITF) had the same basic structure of helix-loop-helix leucine-zipper (bHLH-LZ), which was related to the growth, differentiation and functions of chromocyte [5].

Approximately 60 cases of t(6;11) RCC were reported in the literature, and about 40 of them were genetically confirmed [1, 6-13]. To our knowledge, only 14 cases reported were confirmed the fusion point by RT-PCR and sequencing. Herein, we reported two new cases of t(6;11) RCCs with typical clinicopathologic features as well as molecular/genetic confirmation of the characteristic rearrangement of *TFEB* gene by fluorescence in situ hybridization (FISH) and emphasized a novel *Alpha-TFEB* gene fusion point by RT-PCR and sequencing.

Case report and follow-up

Case 1 was a 38-year-old man, presented at our hospital with complaint of right lumbar pain for two years. Abdominal computed tomography (CT) scan revealed a well-circumscribed heterogeneously mass (13.5 cm × 10.5 cm × 5.3 cm) in the right kidney (**Figure 1A**). Case 2 was a 37-year-old man with a 7.5 cm × 6.7 cm × 5.5 cm cystic-solid lump in the right kidney on the physical examination without other complaints. Other studies, including routine labora-

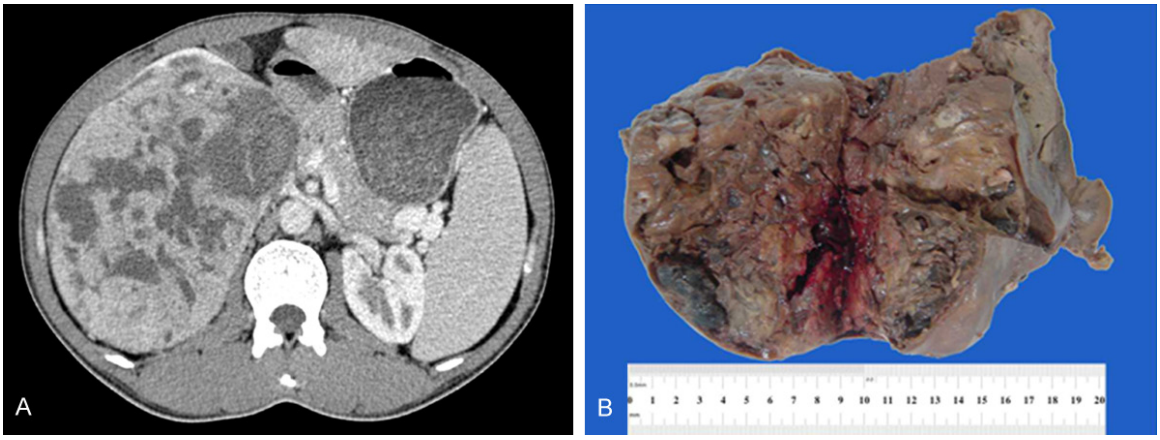

Figure 1. CT image of case 1 showed a heterogeneous well-defined mass accompanied by cystic changes (A). Gross examination of case 1 showed the tumor nearly replaced the whole renal parenchyma, with nodular and cystic, tan-brown colored appearance on cut surface (B).

tory analysis, urinalysis, bone scan and chest X-ray studies were all unremarkable.

Nephrectomy was performed in both cases. No radiotherapy or chemotherapy was administered in either case. Both patients have been closely followed (20 months for case 1 and 16 months for case 2) after surgery, both are doing well with no evidence of local recurrence and distant metastasis.

Materials and methods

Immunohistochemistry

The primary antibodies with the following dilutions were used for immunohistochemistry: TFEB (1:200; Abcam, Cambridge, UK), Melan A (1:50; DakoCytomation, Glostrup, Denmark), HMB45 (1:50; DakoCytomation), TFE3 (1:200; Santa Cruz Biotechnology), EMA (1:100; DakoCytomation), pan-cytokeratin (PCK;1:100; Zymed, San Francisco, CA, USA), CD10 (1:100; DakoCytomation), CK7 (1:300; Zymed). Routine 4-μm sections were prepared from formalin-fixed, paraffin-embedded (FFPE) tissue blocks on 3-aminopropyltriethoxysilane-treated glass slides. Antigen retrieval was achieved by boiling the sections in 0.01 mol/L citrate buffer (pH 6.0) in a high-pressure cooker for 3 min. The Envision kit (DakoCytomation) was used for immunohistochemical staining according to the manufacturer's protocols.

TFEB fluorescence in situ hybridization (FISH)

FFPE tissue sections were examined by using interphase FISH to investigated the rearrangement of the *TFEB* gene region with an LSI dual-color break-apart probe set (provided by Dr. Qiu Rao, Department of Pathology at Nanjing Jinling Hospital, Nanjing, China) [8]. *TFEB* gene rearrangement would result in break-apart of the normal fused green-red signals, resulting in one green/one red break apart signal pattern, usually with one remaining normal green-red fusion signal, rather than the two fused signals as would be seen in a normal cell. Two hundreds interphase nuclei were evaluated. The cut-off value was 10% as previously described [8].

Detection of alpha-TFEB fusion by Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from FFPE tissue block using a miRN easy FFPE Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription was carried out in 20 μL mixture containing 2 μg of total RNA, 4 μL of 5 × Reverse Transcriptate M-MLV buffer (TaKaRa, Shiga, Japan), 1 μL Reverse Transcriptate M-MLV (TaKaRa), 1 μL of oligo $(dT)_{18}$ primer and 2 μL of 10 mM of dNTP mix, for 45 min at 42°C followed by 5 min at 85°C. The primers of *Alpha-TFEB* fusion gene were 5'-GAATAGAGAAGATAGGGA-3' (FP) and 5'-TCTA-AAATGGATGCGGTC-3' (RP). PCR was carried out with Taq HS (TaKaRa, Shiga, Japan): 95°C for 5 min; 40 cycles each consisting of denaturing at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s, and a final extension at 72°C for 10 min. The amplified

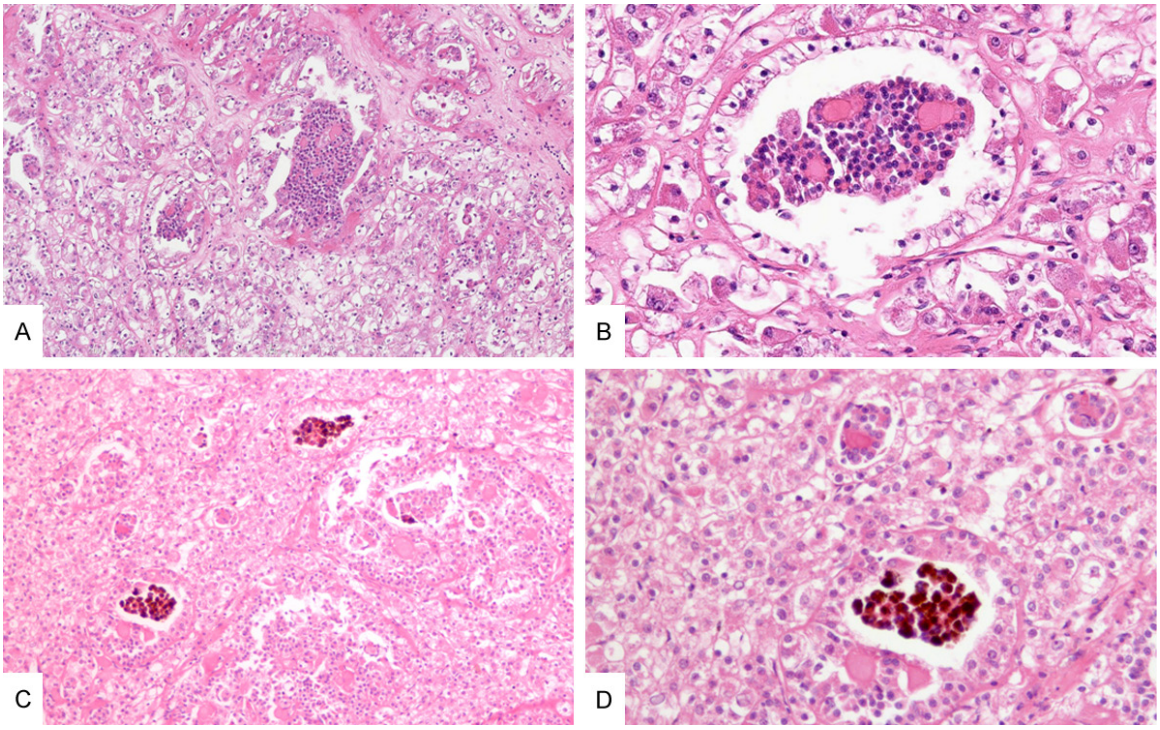

Figure 2. Low (A, C) and high-power (B, D) microscopic fields of case 1 and case 2. Note the typical morphologic features of solid or alveolar architecture, mixture of small and larger cells with clear and eosinophilic cytoplasm. The smaller cells were clustered around hyaline material in the center forming "pseudorosettes" (A-D). Some dark brown pigment was observed in small cell population of case 2 (C, D).

Table 1. Immunohistochemical profile of t(6,11) RCCs in this study

Case	Age (y)\Sex	Immunohistochemical profile							
		TFEB	Melan A	HMB45	TFE3	PCK	EMA	CK7	CD10
1	38\M	+	+	focal+	-	-	-	-	-
2	37\M	+	+	focal+	-	-	-	-	-

fragments were resolved by agarose gel electrophoresis and recovered by gel extraction (Qiagen, Hilden, Germany) and then sequenced.

Results

Morphology

Gross examination: The tumor from case 1 nearly replaced the whole renal parenchyma, with nodular and cystic, tan-brown colored appearance on cut surface. The tumor was confined by the renal capsule, but extended into the renal pelvis (**Figure 1B**). The tumor of case 2 was well circumscribed and the cross-section was yellow-tan, partly cystic and partly solid. No macroscopically noticeable hemorrhage and necrosis was observed in both cases.

Microscopically, tumors showed a characteristically solid or alveolar architecture, mixture of small and larger cells. The large cells had prominent cell membranes, clear or slightly reticulated eosinophilic abundant cytoplasm, and vacuole-like nuclei with small nucleoli. The small cells with narrow eosinophilic cytoplasm, and round or oval nuclei were distinctly clustered around hyaline pink basement membrane material forming "pseudorosettes" (**Figure 2A-D**). Some dark brown pigment was observed in small cell population of case 2 (**Figure 2C**, **2D**). No psammoma bodies were detected in both cases.

Immunohistochemistry

The immunohistochemical findings were summarized in **Table 1**. The tumor cells displayed distinct nuclear staining of TFEB (**Figure 3A**), diffusely cytoplasmic staining of Melan A (**Figure 3B**) and focally positive of HMB45. All the tumor cells showed no staining of TFE3 (**Figure 3C**), CK7, and EMA.

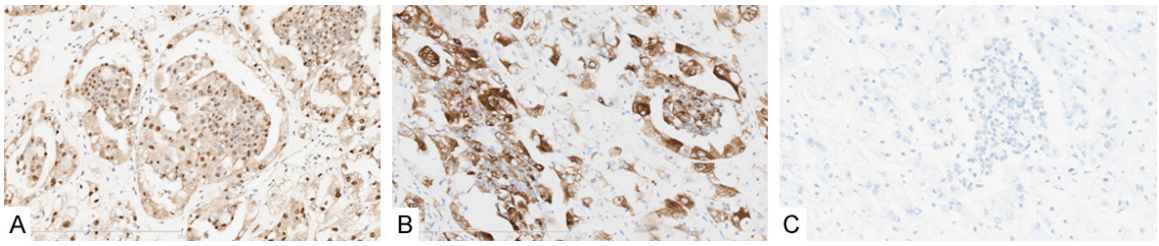

Figure 3. The tumor cells displayed distinct nuclear staining of TFEB (A), diffusely cytoplasmic staining of Melan A (B) and no staining of TFE3 (C).

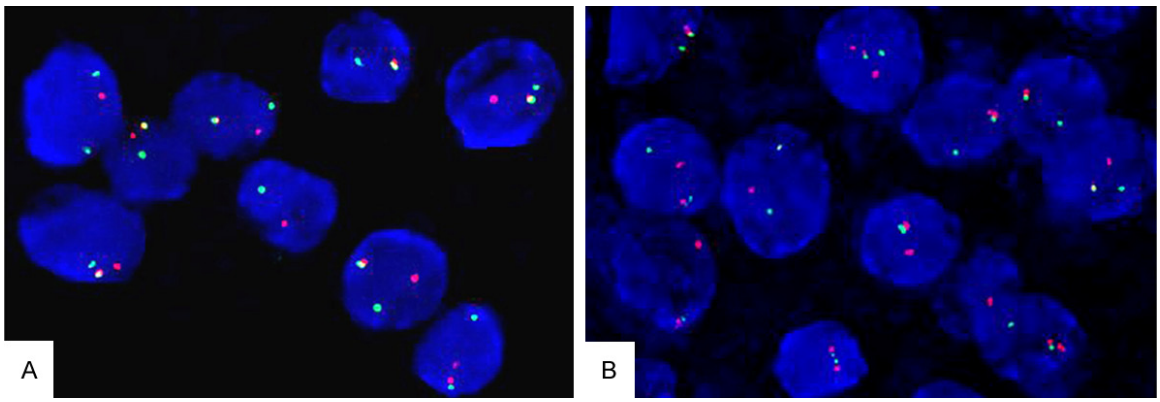

Figure 4. FISH displayed the break-apart one green/one red signal pattern in tumor cells, confirming translocation involving the *TFEB* gene in case 1 (A) and case 2 (B).

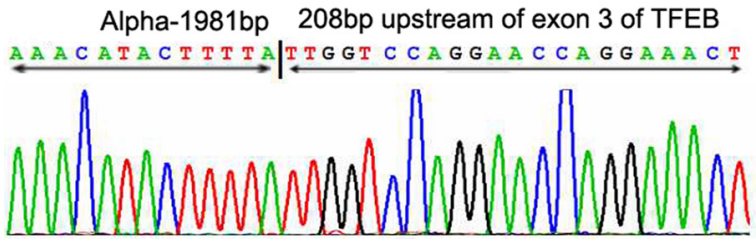

Figure 5. RT-PCR and product sequencing identified a novel fusion point between *Alpha* nucleotide 1981 site and *TFEB* nucleotide 208 site upstream of exon3 in case 1.

TFEB FISH analysis

FISH analysis with the TFEB dual-color break-apart probe showed that 54% (108/200, case 1) and 47% (94/200, case 2) of tumor cell nuclei displayed the characteristic one green/one red break apart signal pattern (**Figure 4**), confirming translocation involving the *TFEB* gene and the diagnosis of t(6;11) RCC.

Detection Alpha-TFEB fusion by RT-PCR

Using the primers described previously [8], a RT-PCR product of 166 bp was detected in case 1. Sequencing identified a novel fusion point between *Alpha* (Genomic Sequence chr11: 65497762-65506469) nucleotide 1981 site and *TFEB* (Genomic Sequence chr6:4168-3980-41735608) nucleotide 208 site upstream of exon3 (**Figure 5**), which was never reported in the literature. No fusion product was identified in case 2 with this primer set.

Discussion

RCCs harboring the t(6;11)(p21;q12) translocation were first described in 2001 [2] and recognized by the ISUP Vancouver classification of renal neoplasia [1] and recently listed in the 2016 WHO classification as a subtype of the MiT family translocation RCC. Patients were of wide age range (3~68 y), but most were young adults (mean age: 28.5 y; median age: 25 y) with a male predominance [6, 14]. Hematuria might be the mainly primary clinical features of this tumor [14]. Some patients discovered the tumor occasionally by physical examination without any symptoms as our case 2.

Table 2. *Alpha-TFEB* fusion point reported in t(6;11) RCCs

Author	Case	Age	Sex	Fusion point	
				Alpha	TFEB
Kuiper et al [4]	1	10	M	1419 bp	96 bp upstream of exon3
	2	20	F	1070 bp	237 bp upstream of exon3
Argani et al [14]	3	18	M	1672 bp	144 bp upstream of exon3
	4	18	F	1580 bp	139 bp upstream of exon3
	5	14	F	1760 bp	162 bp upstream of exon3
	6	9	F	2274 bp	70 bp upstream of exon3
	7	33	M	1795 bp	122 bp upstream of exon3
Pecciarini et al [13]	8	54	F	1631 bp	281 bp upstream of exon3
Zhan et al [11]	9	26	M	1810 bp	94 bp upstream of exon3
Inamura et al [9]	10	57	M	748 bp	235 bp upstream of exon3
	11	37	M	748 bp	42 bp upstream of exon3
	12	46	M	858 bp	79 bp of exon4
Rao et al [8]	13	31	F	1396 bp	118 bp upstream of exon3
	14	30	M	1396 bp	118 bp upstream of exon3
The present study	15	38	M	1981 bp	208 bp upstream of exon3

Histologically, t(6;11) RCCs had a variety of morphologic patterns. The typical morphology of t(6;11) RCC was that of a nested or alveolar epithelioid cell neoplasm with a second population of smaller epithelioid cells associated with hyaline basement membrane material. However, unusual histological features mimicking other RCC subtypes had also been reported, including tumors resembling tubulocystic RCC, chromophobe cell RCC, clear cell RCC, oncocytoma and epithelioid angiomyolipoma et al [7]. Pigment was observed in some cases [8] like our case 2.

Nuclear staining of TFEB was helpful for diagnosis t(6;11) RCC in FFPE tissues. It was different from most other RCCs in that they consistently expressed melanocytic IHC markers such as HMB45 and Melan A but was either negative or only focally positive for epithelial markers. FISH analysis for TFEB rearrangement was the gold standard for diagnosis of t(6;11) RCCs [10].

Because *Alpha* is an intron-less gene and does not contribute to the open reading frame of the *Alpha-TFEB* fusion transcript, the *Alpha-TFEB* fusion points are highly variable, making detection by RT-PCR less reliable than for other gene fusions. So far, only 14 previous cases were genetically analyzed and the reported *Alpha* and *TFEB* fusion points had been summarized in **Table 2**. In case 1 of this study, we identified the translocation breakpoint within intron2 of *TFEB*, just 208 bp upstream of exon3 (protein translation initiates) and the fusion point was different from the previous cases reported.

It was still uncertain about the clinical prognosis of t(6;11) RCCs because of their infrequency. Among the published studies with available treatment and prognosis information, only 3 cases were dead of disease [1]. For our two patients, neither chemotherapy nor radiotherapy was administered after surgery, and no evidence of disease recurrent or progression was found. Therefore, it seems that t(6;11) RCCs have relative indolent biological behavior. However the available data represented a small sample and a short term follow up and may not represent the true nature of these tumors.

In summary, we reported two cases of t(6;11) RCCs, both of them displayed typical morphologic features, immunohistochemical profile and confirmed the diagnosis by FISH analysis. More importantly, we identified a novel fusion point between *Alpha* nucleotide 1981 and *TFEB* nucleotide 208 upstream of exon3 by RT-PCR and product sequencing in case 1.

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Disclosure of conflict of interest

None.

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