Original paper

Clinicopathological study of 5 cases of renal cell carcinoma with t(6;11)(p21;q12)

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Renal cell carcinoma (RCC) with t(6;11)(p21;q12) has been incorporated into the recent WHO classification. We performed a clinicopathological study of 5 cases with such a tumor. The patients consisted of 4 males and 1 female. The age of patients ranged from 17 to 57 years with a mean age of 38.6 years. Tumor sizes ranged from 2.8 to 11 cm with a mean value of 6.5 cm. Despite immunotherapy and molecular-targeted therapy, one patient died of the disease 28 months after the surgery. Grossly, the cut surface of this tumor showed grayish white color in at least the focal area of all tumors. Furthermore, hemorrhage, daughter nodules and cystic changes were observed in two, three, and two tumors, respectively. Morphologically, all the tumors consisted of two components of large cells and small cells, and the latter surrounded basement membrane-like materials, forming rosette-like structures. Immunohistochemically, nuclei of tumor cells in all cases were positive for TFEB. Fluorescence *in situ* hybridization study confirmed the *TFEB* gene break in two tumors. Finally, urologists and pathologists should bear in mind that this tumor may occur in young adults to adults and might behave in an aggressive fashion. Break-apart FISH is useful for the definite diagnosis.

Key words: t(6;11), renal cell carcinoma, TFEB.

Introduction

The new category of MiT/TFE family translocation renal cell carcinoma (RCC) has been recently proposed and incorporated into the recent World Health Organization classification in 2016 [1, 2, 3, 4, 5]. The MiT/TFE family RCC chiefly consists of Xp11.2 RCC and t(6;11) RCC showing the overexpression of TFE3 and TFEB protein. However, reported cases of t(6;11) RCC are still limited. We performed a clinicopathological study of 5 Japanese cases with t(6;11) RCC to elucidate the clinicopathological characteristics.

Table I. Clinical characteristics [1]

Case	Age	Sex	Site	Symptom/reason for discovery
1	17	F	L, lower	acute abdomen
2	45	M	L, upper	during further examination of other disease
3	40	M	R, upper	during medical check-up
4	57	M	L, upper	during further examination of other disease
5	34	M	R, middle	right subcostal pain

F – female; M – male; L – left; R – right

Material and methods

Among 655 cases with renal tumors consulted in the Department of Diagnostic Pathology, Kochi Red Cross Hospital during January 2010 and December 2015, 5 cases with t(6;11) RCC were selected in this study. In this study, the clinicopathologic characteristics were predominantly analyzed with the focus on symptoms, follow-up data, macroscopic and microscopic findings, immunohistochemical findings and molecular genetic findings. Histologic sections were cut from formalin-fixed paraffin-embedded tissue blocks and stained with hematoxylin and eosin. For the immunohistochemistry, tissue sections were cut and stained with Ventana Benchmark XT or Ultra autostainer (Ventana Medical Systems, Tucson, AZ). Primary antibodies against TFEB (polyclonal, V-17, 1 : 400, Santa Cruz Biotechnology, Inc., Dallas, TX), TFE3 (MRQ-37, prediluted, Ventana Medical Systems, Inc., Tucson, AZ), cathepsin-K (3F9, 1 : 6400, Abcam, Tokyo, Japan), melanosome-related antigen (MRA) (HMB45, prediluted, DAKO, Glostrup, Denmark), Melan A (A103, 1 : 100, Novocastra Laboratories Ltd, Newcastle upon Tyne NE 128EW, UK), CD10 (56C16, prediluted, Novocastra Laboratories Ltd, Newcastle upon Tyne NE 128EW, UK) and AMACR (P504S)(13H4, 1 : 100, DAKO, Glostrup, Denmark) were employed in the present study. Fluorescence *in situ* hybridization (FISH) was performed using the *TFEB* gene Break Apart Probe (Empire Genomics, New York, USA). Pretreatment using VP-2000 Processor (Abott Molecular, Tokyo, Japan) was performed according to the manufacturer's protocol for HER2, and hybridization was carried out using ThermoBrite (Abott Molecular, Tokyo, Japan). However, the hand-driven method was adopted for the immunohistochemistry of TFEB protein. FISH signals were observed using the Axio Imager 2 Upright Microscope (Zeiss, Tokyo, Japan) and analyzed using the MetaCyte Lite (MetaSystems, Altlussheim, Germany) with Isis (Zeiss). The positive cut-off value of the separate signals between red and green in FISH results was considered as more than 10%. This study was approved by the ethical committee of Kochi Red Cross Hospital (number 143).

Results

Patient information and symptoms/reason for discovery

The results are summarized in Table I. The patients consisted of 4 males and 1 female. The age of patients ranged from 17 to 57 years with a mean age of 38.6 years. One patient presented with acute abdomen because of severe hemorrhage due to capsular rupture of the tumor, and one patient complained of right subcostal pain. One patient and two patients were incidentally found during medical check-up and further examinations of other diseases.

Imaging analyses

Ultrasound sonography and computed tomography (CT) scan showed a well-circumscribed tumor. In two cases, contrast dynamic CT scan of the abdomen revealed heterogeneous enhancement in the renal tumorous lesion. Magnetic resonance imaging showed high intensity in T1 and low intensity in T2 in one tumor (case 2). FDG-PET was not available in all tumors.

Tumor size and pathological stage

The results are summarized in Table II. The size ranged from 2.8 to 11 cm with a mean of 6.5 cm. Two patients, one patient, one patient and one patient were of stages I, II, III and IV, respectively.

Postoperative therapy and follow-up data

The results are summarized in Table III. Regarding postoperative therapy, three patients received

Table II. Clinical characteristics [2]

Case	Tumor size	Stage
1	11 cm	III (pT3aN0M0)
2	6 cm	IV (pT3aN1M1)
3	9 cm	II (pT2N0M0)
4	2.8 cm	I (pT1aN0M0)
5	3.5 cm	I (pT1aN0M0)

Table III. Therapy and outcome

Case	Postoperative therapy	Follow-up duration	Outcome
1	NP	NI	NI
2	IFN-α, VEGFR/ PDGFR-I (sunitinib), mTOR-I (temsirolimus), radiation	28 months	DOD
3	IFN-α	84 months	AWOD
4	NP	19 months	DOAD
5	NP	recent case	recent case

NP – not performed; INF – interferon; NP – not performed; INF – interferon; mTOR-I – mammalian target of rapamycin inhibitor; NI – not informative; DOD – died of disease; AWOD – alive without disease; DOAD – died of another disease

Table IV. Macroscopic findings

Case	Color	H	N	DN	C
1	grayish white	+	–	+	–
2	grayish white	+	–	+	–
3	grayish white	–	–	+	+
4	brown/grayish white	–	–	–	–
5	grayish white/yellow	–	–	–	+

H – hemorrhage; N – necrosis; DN – daughter nodule; C – cyst

no additional therapies. Case 2 received interferon-α (IFN-α), vascular endothelial growth factor receptor/platelet-derived growth factor receptor (VEGFR/PDGFR) inhibitor (sunitinib) and mammalian target of rapamycin (mTOR) inhibitor (temsirolimus), case 3 underwent IFN-α therapy. Regarding outcome, case 5 was very recent and the follow-up data of case 1 was not available. In the remaining three cases, the follow-up duration ranged from 19 to 84 months with a mean of 43.7 months. Although the case 2 patient received IFN-α, VEGFR/PDGFR inhibitor and mTOR inhibitor, he died of the disease after 28 months of follow-up. The case 3 patient who received IFN-α therapy is alive without disease with 84 months of follow-up. The case 4 patient received hemodialysis postoperatively and died of another unrelated disease with 19 months follow-up.

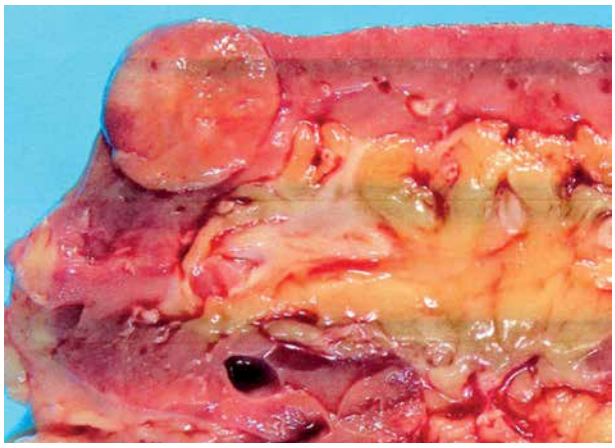

Fig. 1. Macroscopic findings of case 4. The cut surface shows brown to grayish white color with solid consistency

Macroscopic findings

The results are summarized in Table IV. The cut surface of the tumor showed gray color in three tumors, grayish white to yellow color in one tumor, and brown to grayish-white color in one tumor (Fig. 1). Hemorrhage was identified in two tumors, while no necrosis was seen in any tumors. Daughter nodules and cystic formation were observed in three tumors and two tumors, respectively.

Microscopic findings

The results are summarized in Table V. Histologically, the tumor predominantly showed solid to alveolar growth pattern of neoplastic cells with eosinophilic and clear cytoplasm (Fig. 2A). Additionally, the tumor consisted of the two components of large cells and small cells in all tumors (Fig. 2B). However, small cells were considerably scant in case 4. Basement membrane-like materials were observed in the small cell area with a round pattern, resulting in rosette-like structures (Fig. 2B). In one tumor (case 4), rosette-like structures were indistinct, because the number of small cells were very limited. Papillary architectures were present in four tumors (Fig. 2C). A proliferating area of tubules with eosinophilic to oncocytic cytoplasm was observed in one tumor (Fig. 2D). Mitotic figures and angiolymphatic invasion were observed in one tumor (case 2). Psammoma bodies were

Table V. Morphological findings

Case	*two-cell pattern	PR	papillary	PB	ET
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	–
4	small cells are scant	indistinct	–	–	–
5	+	+	+	-	+

PR – pseudorosettes; PB – psammoma bodies; ET – entrapped tubules
**Two cells mean large and small cells*

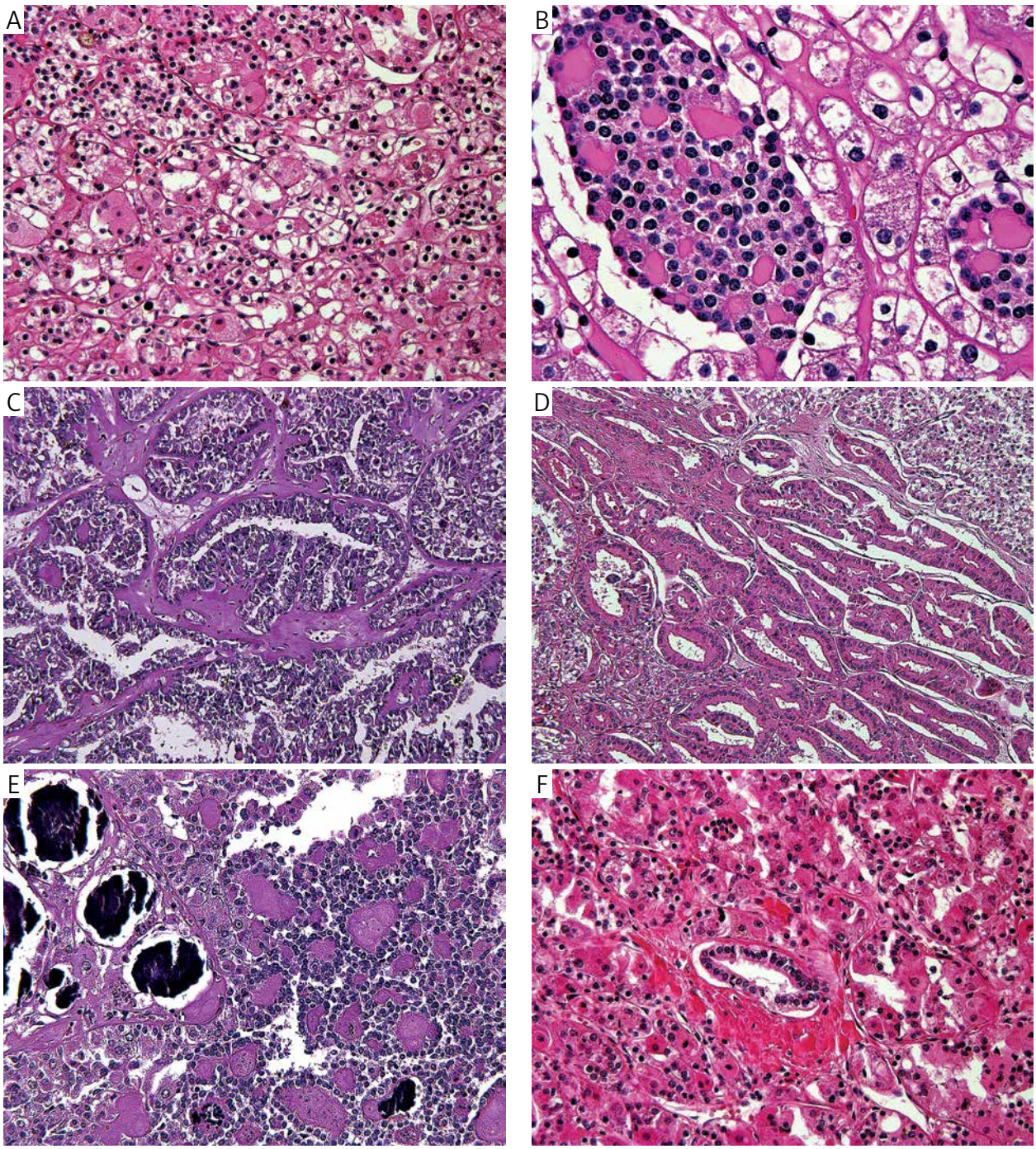

Fig. 2. Microscopic findings. A) The tumor shows solid to alveolar growth pattern of neoplastic cells admixed with eosinophilic and clear cytoplasm. B) The tumor consists of both large and small neoplastic cells surrounding basement membrane materials, resulting in pseudorosette formation. C) Papillary growth pattern. D) Unusual tubular growth with eosinophilic to oncocytic. E) Psammoma bodies are noted in the stroma. F) An entrapped tubule is seen in the tumor-center stroma

Table VI. Immunohistochemical findings

Case	cathepsin-K	MRA	Melan A	TFEB	TFE3	CD10	AMACR
1	d+	f+	d+	d+	–	f+	f+
2	d+	f+	d+	d+	–	–	f+
3	f+	-	f+	d+	–	f+	f+
4	f+	-	f+	f+	–	NP	NP
5	d+	-	d+	d+	–	NP	NP

d – diffuse; f – focal; NP – not performed; + positive; – negative; MRA – melanosome-related antigen (HMB45)

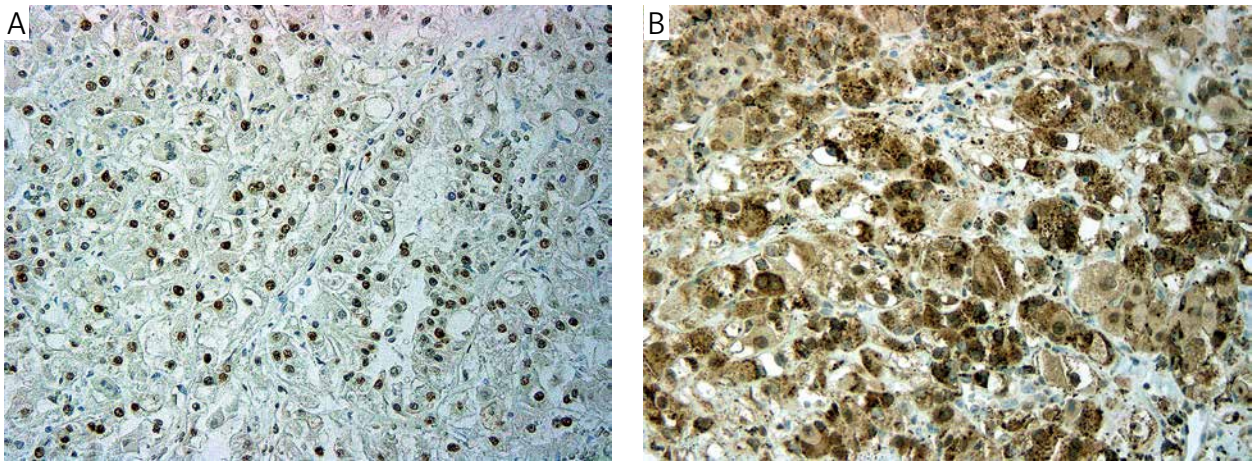

Fig. 3. Immunohistochemical findings. A) Nuclei of many neoplastic cells show positivity for TFEB protein. B) Cathepsin-K is diffusely immunoreactive

observed in the stroma of three tumors (Fig. 2E). Additionally, entrapped tubules in the tumor-center stroma were identified in three tumors (Fig. 2F). No infiltrating lymphocytes were identified in the tumorous stroma.

Immunohistochemical findings

The results are summarized in Table VI. All the tumors showed positivity for TFEB (Fig. 3A). Four tumors showed diffuse positivity with more than 2+ intensity, whereas one tumor showed focal positivity with 1+ intensity. At contrast, all tumors showed negative results for TFE3. Cathepsin-K was positive in all tumors with diffuse distribution in three tumors and focal distribution in two tumors (Fig. 3B). Melanosome-related antigen (MRA) (HMB45) was focally positive in two tumors. The remaining three tumors were completely negative for MRA. Melan A was positive in all tumors with diffuse distribution in three and focal localization in the remaining two tumors. CD10 was focally positive in two tumors and AMACR was focally positive in three tumors.

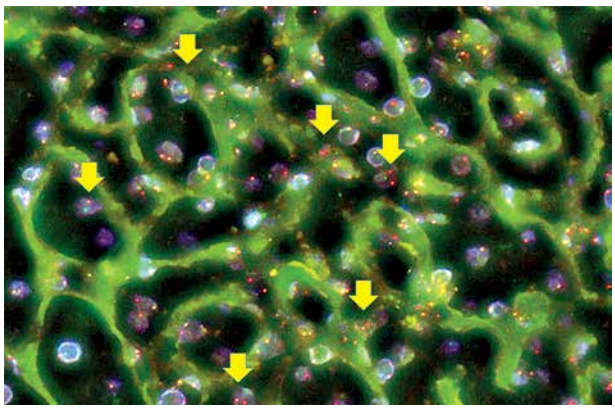

Fig. 4. Fluorescence *in situ* hybridization for *TFEB* gene. Split signals are observed (arrows)

Molecular genetic findings

In *TFEB* gene break-apart FISH analyses of two tumors, *TFEB* gene splitting was observed (Fig. 4). In case 4, 212 tumor cells were counted and showed positive signals in 42%. In case 5, 108 tumor cells were counted and showed positive signals in 62.5%.

Discussion

RCC with t(6;11)(p21;q12) has been incorporated into the MiT translocation carcinoma of the recent WHO classification [1, 2, 3, 4, 5]. As this tumor generally occurs in children and young adults, urologists and pathologists should take the differential diagnosis of renal tumor arising in children and young adults into consideration [6, 7, 8, 9, 10, 11, 12, 13]. As

observed in the present study, this tumor may occur even at the age over 40 years [14, 15, 16, 17, 18, 19, 20]. In some cases, previous cytotoxic chemotherapy may be involved in the pathogenesis of this tumor [8]. Therefore, the presence of a past history is very important for urologists. Regarding the reason for discovery, this tumor seems to be often found during the medical check-up or further examination of other diseases. Macroscopically, this tumor is often found at a large size, because this tumor often occurs in young adults and these patients have no chance receiving a medical check-up until the renal tumor is discovered. Based on the results of gross findings, grayish-white color and presence of hemorrhage, cyst and daughter nodules seem to be characteristic of this tumor. A tubulocystic pattern was also reported previously, but this phenomenon seems to be very rare [20]. Histologically, this tumor is chiefly composed of two kinds of neoplastic cells of both large cells resembling usual clear cell RCC cells and small cells simulating lymphocytes [21, 22, 23, 24]. This finding seems to be a most important diagnostic clue. However, small cells may be indistinct or absent in some cases, as observed in one tumor of the present study [12, 25]. For this reason, pathologists should try extensive tumor sampling at the gross examination or seek the small cell area deliberately at the histological examination whenever RCC with t(6;11)(p21;q12) is clinically and histologically suspected. Psammoma bodies in the stroma seem to be often observed in RCC with t(6;11)(p21;q12) as well as Xp11.2 RCC [26]. This tumor shows overexpression of TFEB protein because of the formation of the *Alpha-TFEB* chimeric transcript caused by chromosomal translocation between 6p21 and 11q12, and nuclear expression of TFEB is a highly sensitive and specific diagnostic marker [7]. The combined immunohistochemical stainings for cathepsin K, melanosome-related antigen (clone HMB45) and melan A seem to be an adjunctive diagnostic tool in identification of RCC with t(6;11)(p21;q12) [4, 6, 7, 11, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24, 27, 28]. The break-apart FISH study for the *TFEB* gene seems to be an excellent measure for the definite diagnosis because the fusion partner for the *TFEB* gene is limited to the *Alpha* (*MALAT*) gene [17, 20, 23, 28, 29]. This fact essentially seems to differ from that of Xp11.2/*TFE3* translocation-associated RCC having several fusion partners [1, 2, 3, 4, 5, 21, 30]. Cytogenetics and RT-PCR analyses are also available for the detection of this tumor [6, 7, 14, 16, 18, 31]. The prognosis seems to be generally excellent [10, 11, 18, 27, 30] with several exceptions. Some tumors did cause recurrence, metastasis or a dismal outcome, particularly in adult cases [15, 16, 25]. In metastatic tumors, mTOR inhibitor may be effective because this tumor is nowadays considered to be related to the mTOR pathway [29]. Immune therapy such as interferon and a VEGF/PDGF inhibitor such as sunitinib may be valuable in some cases [15]. In this viewpoint, evaluation of the vascular pattern in RCC with t(6;11)(p21;q12) may be important, as some histological subtypes including clear cell, papillary, chromophobe and unclassified RCCs were analyzed [31]. Anti-immune check point agents could be hopeful. As downregulation of miR-630 can inhibit the tumor progression in RCC, miR-630 may be a potential therapeutic target also in RCC with t(6;11)(p21;q12) [32, 33].

In conclusion, urologists and pathologists need to consider RCC with t(6;11)(p21;q12) when the patients are children and young adults, and the tumor shows a grayish-white color with hemorrhage, cyst and daughter nodules. In such a situation, the immunohistochemistry of TFEB protein and break-apart FISH for the *TFEB* gene may be available for an accurate diagnosis.

The authors declare no conflicts of interest.

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