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Original Article

Immunohistochemical characterization of renal tumors in patients with Birt-Hogg-Dubé Syndrome

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Birt-Hogg-Dubé syndrome (BHD) is an autosomal dominant disorder associated with a germline mutation of folliculin (*FLCN*). The affected families are at a high risk for developing multiple renal cell carcinomas (RCC). Little is known about the immunostaining patterns of mutant *FLCN*-associated RCCs. We investigated 32 RCCs obtained from 17 BHD patients. The studied tumors included chromophobe RCCs (*n* = 15), hybrid oncocytic/chromophobe tumors (HOCT) (*n* = 14) and clear cell RCCs (*n* = 3). Almost all chromophobe RCCs and HOCTs revealed positive staining for S100A1, Ksp-cadherin and CD82. They stained either focally or diffusely for CK7, and were negative for CA-IX. All clear cell RCCs were positively stained for CA-IX and negative for CK7. These data confirmed that mutant *FLCN*-associated oncocytic and clear cell RCCs exhibited generally similar immunostaining patterns compared to their sporadic counterparts. Frequent positive staining for S100A1, Ksp-cadherin and CD82 in chromophobe RCCs and HOCTs indicated that these two types were relatively similar rather than distinctively different in their patterns of immunoreactivity. Characteristic peri-nuclear halos and polygonal cells with clear cytoplasm, which often misleads pathologists into the diagnosis of clear cell RCC, should be carefully examined using an immunohistochemical panel including CA-IX, Ksp-cadherin, CD82 and CK7.

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Renal cell carcinomas (RCCs) occasionally develop in patients with familial cancer syndrome.[1] Some types of hereditary RCC exhibit disease-associated histopathological features. For example, patients with von Hippel-Lindau disease have a risk for developing clear cell RCC. Patients with tuberous sclerosis complex often have renal angiomyolipoma. Birt-Hogg-Dubé syndrome (BHD), also called Hornstein-Knickenberg syndrome, is an inherited disorder characterized by multiple renal tumors,[2,3] in which the responsible gene is folliculin (*FLCN*).[4] In the majority of renal tumors of BHD patients, the oncocytic cells possess perinuclear halos and occasionally demonstrate complicated features composed of two or more histological types.[5,6] FLCN is thought to function as a tumor suppressor. It has recently been determined to be a key factor in mitochondrial oxidative metabolism.[7,8]

The number of published histopathological studies of human renal tumors from BHD is quite limited.[5,6,9,10] The landmark study using the largest number of clinical cases was performed by Pavlovich *et al.* in 2002, who described 130 tumors from 30 BHD patients.[6] In their study, hybrid oncocytic/chromophobe tumors (HOCT) were the most frequent histological type. Since both oncocytic and clear cell type renal tumors can develop in patients with BHD, pathologists find it difficult to classify these renal tumors solely by microscopic findings especially when information about genetic background is not provided. Immunohistochemical

studies have not been performed using a substantial number of genetically confirmed tumors.

In the present study, we investigated the immunohistochemical features of 32 renal tumors in which the genetic mutation patterns were determined. We discuss immunohistochemical findings and their significance in diagnosing renal tumors associated with BHD. Since the World Health Organization's renal tumor classification does not yet give an official name to renal tumors in patients with BHD, in this study we provisionally call them mutant *FLCN*-associated renal tumors.

MATERIALS AND METHODS

Samples

Thirty-two renal tumors from 17 patients (16 families) were enrolled in this study (Table 1). Written informed consent was obtained from each patient. The study was approved by the institutional review board of Yokohama City University. Resected renal tissues were fixed with 10% buffered formalin and embedded in paraffin. Hematoxylin and eosin (HE) staining was performed for histological diagnosis. Sporadic chro-

Table 1 Histological and genetic information

Patient (P) -Tumor (T)	Gender (age*)	Chief complaint, other symptoms	Histology (stage)	Diameter (mm)	*FLCN* germline mutation pattern
P1-T1	F	Familial RCCs,	HOCT (I)	25	Exon 5 c. 332_349delACCCC
P1-T2	(46)	PCs	HOCT (I)	12	-AGCCACCCCCAGC
P1-T3			HOCT (I)	4	
P2-T1	M (67)	Unusual histology of RCC,PCs	HOCT (I)	21	Exon 13 c.1533_1536 delGATG
P3-T1	F	Bilateral RCCs,	Chromo (I)	30	Exon 11 c.1285dupC
P3-T2	(68)	PCs/ skin papules	HOCT (I)	10	
P4-T1	F (45)	Bilateral RCCs,PTXs/ PCs	HOCT (I)	46	Exon 11 c.1285dupC
P5-T1	M	Bilateral RCCs,	Chromo (I)	26	Exon 9 c.906dupT
P5-T2	(48)	PTXs/ PCs	Chromo (I)	14	
P6-T1	F (29)	Familial PTXs, PCs	HOCT (I)	28	Exon 11 c.1285dupC
P7-T1	M (63)	Unusual histology of RCC,PCs	Chromo (I)	11	Exon 12c.1347_1353 dupCCACCCT
P8-T1	M (45)	Unusual histology of RCC,PCs	HOCT (I)	26	Exon 13 c.1533_1536 delGATG
P9-T1	F	Bilateral RCCs,	Chromo (I)	31	Exon 11 c.1285dupC
P9-T2	(49)	PCs	Chromo (I)	13	
P10-T1	M (54)	Detection of RCC in medical check,PCs / skin papules	Chromo (I)	24	Exon 11 c.1285dupC
P11-T1	F (40)	Detection of RCC in medical check, PCs	Clear cell (I)	40	Exon 12c.1347_1353 dupCCACCCT
P12-T1	M	Bilateral RCCs,	Chromo (I)	20	Exon 13 c.1533_1536 delGATG
P12-T2	(57)	PCs/ familial PTXs	HOCT (I)	13	
P13-T1	F (72)	Familial RCCs,PCs/ skin papules	HOCT (I)	12	Exon 13 c.1533_1536 delGATG
P14-T1	F	Bilateral RCCs,	Chromo (I)	43	Exon 4c.199dupG
P14-T2	(67)	PCs/ skin papules	Chromo (I)	37	
P14-T3		/ familial RCCs	HOCT (I)	20	
P15-T1	M	Bilateral RCCs,	Clear cell (III)	80	Exon 11 c.1285dupC
P15-T2	(58)	PTXs/ PCs	HOCT (I)	20	
P16-T1	M	Bilateral RCCs,	Chromo (I)	43	Exon 13 c.1528_1530 delGAG
P16-T2	(50)	PTXs/ PCs	Chromo (I)	14	
P16-T3		/ skin papules	Chromo (I)	8	
P16-T4			Chromo (I)	9	
P16-T5			Chromo (I)	17	
P16-T6			HOCT (I)	32	
P16-T7			HOCT (I)	10	
P17-T1	M (61)	PTXs, PCs/ familial PTXs	Clear cell (I)	25	Exon 11 c.1285dupC

Age*: The age of initial RCC detection.

Chromo, chromophobe renal cell carcinoma; Clear cell, clear cell renal cell carcinoma; F, female; HOCT, hybrid oncocytic/chromophobe tumor; M, male; PC, pulmonary cyst; PTX, pneumothorax; RCC, renal cell carcinoma.

Table 2 Sources and antibody dilutions

Antibody specificity to	Source	Working dilution
Cytokeratin 7	DAKO (Glostrup, Denmark)	1:50
CD82	Santa Cruz (Santa Cruz, CA)	1:100
Ksp-cadherin	Invitrogen (San Diego, CA)	1:100
S100A1	Novus (Littleton, CO)	1:100
Carbonic anhydrase IX	Abcam (Cambridge, United Kingdom)	1:50

mophobe RCCs (n = 11), oncocytomas (n = 11) and clear cell RCCs (n = 12) were used for comparison. Patients with sporadic renal tumors did not receive genetic testing for *FLCN*, but none had a past history of pneumothorax, pulmonary cysts or fibrofolliculomas. Histological types were determined by two pathologists with expertise in renal tumors (N. Kuroda and Y. Nagashima).

Immunohistochemistry

Paraffin sections of 4-μm thickness were subjected to immunohistochemistry. After deparaffinization and rehydration, sections were autoclaved at 121°C for 15 min, after which they were treated with the diluted antibodies at 4°C overnight. Immunohistochemical staining was done using the EnVision$^+$ kit (Dako, Glostrup, Denmark), followed by 3, 3'-diaminobenzidne staining for visualization. The sources and dilutions of antibodies are shown in Table 2. The intensity of the immunostaining was semi-quantitatively graded as follows: (–), < 5% of cells positive; (+), 5 to 10% of cells positive; (++), > 10% of cells positive.

DNA isolation

DNA from peripheral blood leukocytes was obtained using the LabPass Blood Mini kit (Cosmo GENETECH, Seoul, Korea) according to the manufacturer's instructions.

Direct sequencing

Exons 4–14 of the *FLCN* gene were amplified by PCR using the primers described previously.[4] PCR conditions were described in our previous study.[11] After purification, DNA was labeled with the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Bedford MA) and DNA sequencing was done using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Statistical analysis

Statistical analysis was performed with JMP11 software (SAS Institute, Cary, NC). The Chi square test was used when comparing groups. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Genetic mutations, histological types and tumor sizes

The histological types, the tumor sizes and *FLCN* germline mutation patterns are summarized in Table 1. All patients were Japanese, and the group was composed of 8 females and 9 males. P9 and P10 were siblings. Other patients had no kinship with one another. All patients underwent genetic testing and were found to have heterozygous frameshift mutations in *FLCN*. The duplication of cytosine in the C8 tract in exon 11 (c.1285dupC) was the most frequent germline mutation pattern. It was detected in seven patients. Deletion of GATG in exon 13 (c.1533_1536delGATG) was detected in four patients and duplication of CCACCCT in exon 12 (c.1347_1353dupCCACCCT) was detected in two patients. Other affected patients had different germline mutation patterns. Fifteen tumors were diagnosed as chromophobe RCC and 14 were diagnosed as HOCT (Fig. 1a–d). Three tumors were diagnosed as clear cell RCC (Fig. 2a–d). Tumor diameters were between 4 and 80 mm. All tumors, except for P15-T1, were stage I. None had lymphovascular space invasion. P15-T1, a stage III clear cell RCC, had necrosis and peri-renal fat invasion. Statistical analysis revealed no significant correlation in tumor size or mutation pattern between chromophobe RCC and HOCT (data not shown).

Immunohistochemical findings of solid tumor areas

The results of immunostaining of mutant *FLCN*-associated tumors and sporadic tumors are shown in Table 3. Immunostaining for Ksp-cadherin and CK7, which are markers of distal tubules and collecting ducts,[12,13] was performed to estimate tumor origin. Immunostaining for CD82 and S100A1 was performed to characterize immunohistochemical features of oncocytic cells with perinuclear halos as either chromophobe RCC-like or oncocytoma-like.[12]

More than 10% of tumor cells stained positively for Ksp-cadherin in all HOCTs (Fig. 1e) and 11 of 14 chromophobe RCCs. Four chromophobe RCCs showed positive staining for Ksp-cadherin in limited areas. One of three clear cell RCCs showed focal immunoreactivity, and two were negatively stained for Ksp-cadherin.

All chromophobe RCCs were positive for CK7, although seven of 14 cases showed focal immunoreactivity (Fig. 1f). Four HOCTs and all three clear cell RCCs were negative for CK7.

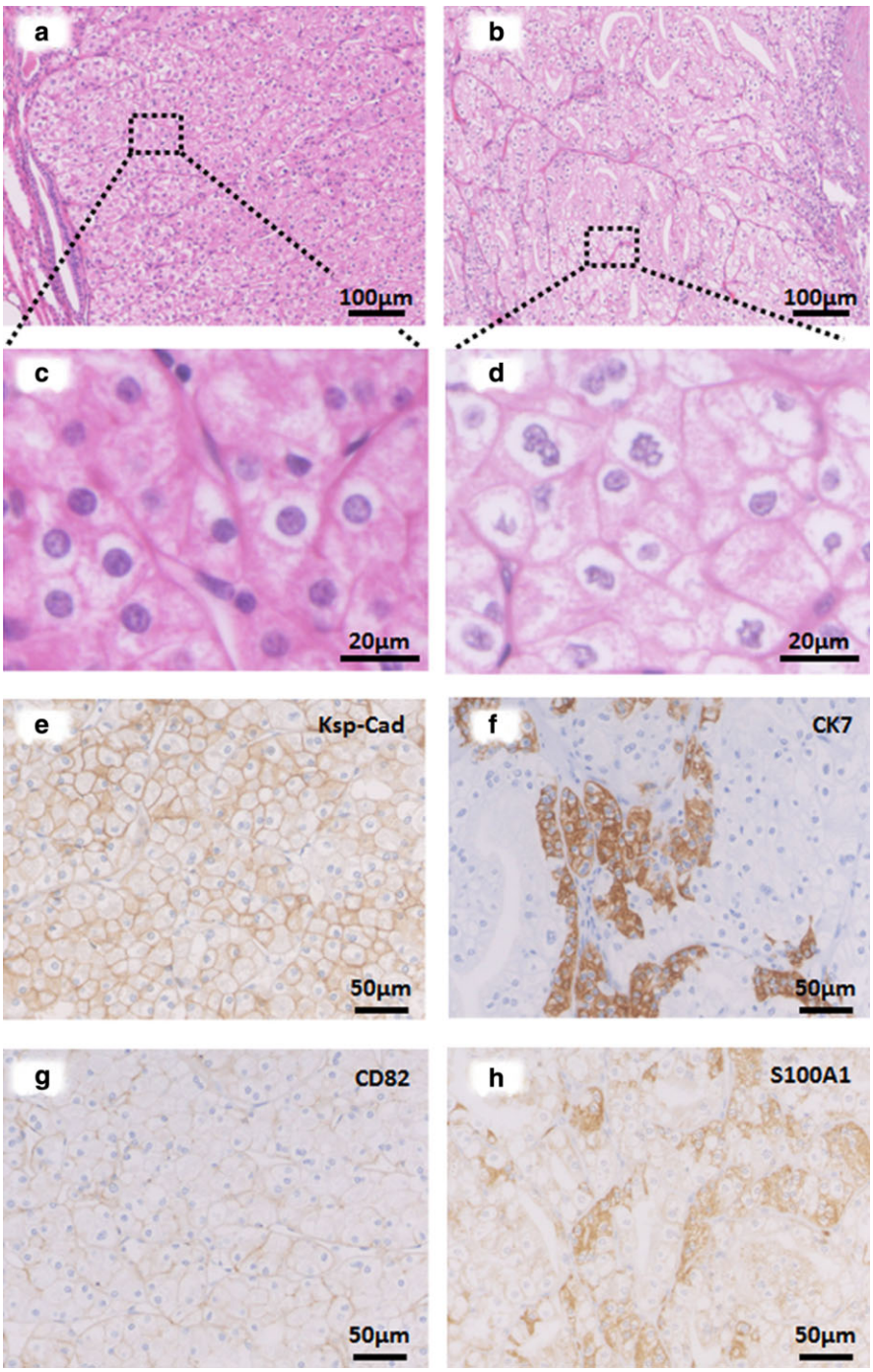

Figure 1 Histological findings of oncocytic mutant *FLCN*-associated renal tumors. (**a–d**) Hematoxylin and eosin staining of (**a**, **c**) a hybrid oncocytic/chromophobe tumor in P2-T1 and (**b**, **d**) a chromophobe renal cell carcinoma in P9-T1. Cells in the hybrid oncocytic/chromophobe tumor had oncocytoma-like granular cytoplasmas and round nuclei, and simultaneously had chromophobe renal cell carcinoma-like distinctive cell-cell boundaries and perinuclear halos. (**e, g**) Immunostaining for (**e**) Ksp-cadherin and (**g**) CD82 in a hybrid oncocytic/chromophobe tumor (P2-T1). (**f, h**) Immunostaining for (**f**) CK 7 and (**h**) S100A1 in a chromophobe renal cell carcinoma (P9-T1).

More than 10% of tumor cells were positively stained for CD82 in 10 of 14 chromophobe RCCs and 13 of 15 HOCTs (Fig. 1g). A few cases in each group were negatively stained or focally stained. Clear cell RCCs were either focally (1 of 3) or negatively (2 of 3) stained.

The majority of HOCTs showed diffuse staining for S100A1 (11 of 13). In addition, 13 of 14 chromophobe RCCs demonstrated either focal or diffuse immunoreactivity for S100A1 (Fig. 1h).

CA-IX, an immunostaining marker for clear cell RCC,[12] stained positively in all three clear cell RCCs (Fig. 2d) and negatively stained in other types of tumors. In one HOCT, a few papillary glands were focally stained for CA-IX.

Immunostaining patterns of sporadic renal tumors were examined for comparison (Table 3). All 11 chromophobe RCCs were diffusely positive for CD82 (100%), whereas two of 11 oncocytomas were positive for CD82 (18.1%). Nine of 11 (81.8%) chromophobe RCCs were diffusely stained for

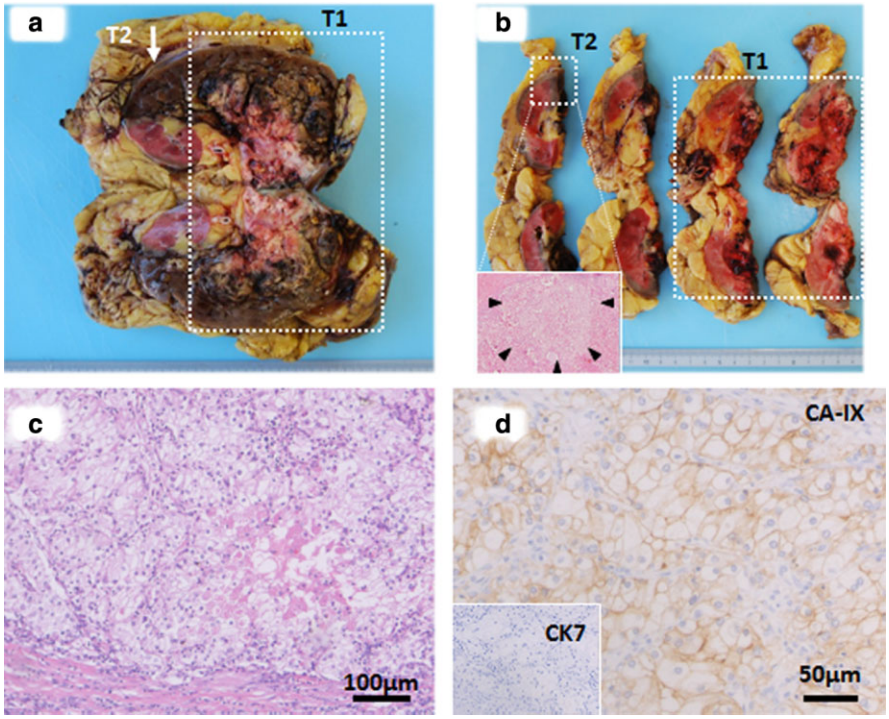

Figure 2 Macroscopic and histological findings of a mutant *FLCN*-associated clear cell renal cell carcinoma. (**a**) The resected right kidney in P15. The yellowish-white tumor with bleeding is T1 (a dotted rectangle). A tiny nodule (T2) is indicated by an arrow. (**b**) The sectioned surfaces of the right kidney in P15. T1 and T2 are separately located. Inset: Microscopic feature of T2 (HOCT). (**c**) Hematoxylin and eosin staining of clear cell renal cell carcinoma in P15-T1. (**d**) Tumor cells show positive staining for CA-IX in P15-T1. Inset: Negative staining for CK7 in a serial section.

Table 3 Summary of immunostaining of mutant *FLCN*-associated and sporadic tumors

	Mutant *FLCN*-associated tumors			Sporadic tumors		
	HOCT	chRCC	ccRCC	Oncocytoma	chRCC	ccRCC
Immunostaining	*n* = 14 (%)	*n* = 15 (%)	*n* = 3 (%)	*n* = 11 (%)	*n* = 11 (%)	*n* = 12 (%)
Ksp-cadherin						
–	0 (0)	0 (0)	2 (66.7)	5 (45.5)	0 (0)	12 (100)
+	0 (0)	4 (26.7)	1 (33.3)	5 (45.5)	0 (0)	0 (0)
++	14 (100)	11 (73.3)	0 (0)	1 (9.0)	11 (100)	0 (0)
CK7						
–	4 (30.8)	0 (0)	3 (100)	7 (63.6)	2 (18.2)	12 (100)
+	5 (38.4)	7 (50)	0 (0)	3 (27.3)	0 (0)	0 (0)
++	4 (30.8)	7 (50)	0 (0)	1 (9.1)	9 (81.8)	0 (0)
NE	1	1	0	0	0	0
CD82						
–	2 (14.3)	1 (6.7)	2 (66.7)	9 (81.8)	0 (0)	12 (100)
+	2 (14.3)	1 (6.7)	1 (33.3)	0 (0)	0 (0)	0 (0)
++	10 (71.4)	13 (86.6)	0 (0)	2 (18.2)	11 (10 0)	0 (0)
S100A1						
–	0 (0)	1 (6.7)	0 (0)	0 (0)	4 (36.4)	0 (0)
+	2 (15.4)	6 (40)	0 (0)	0 (0)	5 (45.4)	0 (0)
++	11 (84.6)	8 (53.3)	3 (100)	11 (100)	2 (18.2)	12 (100)
NE	1	0	0	0	0	0
CA-IX						
–	13 (100)	15 (100)	0 (0)	11 (100)	11 (100)	0 (0)
+	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	1 (8.3)
++	0 (0)	0 (0)	2 (66.7)	0 (0)	0 (0)	11 (91.7)
NE	1	0	0	0	0	0

Mutant *FLCN*-associated HOCTs and sporadic oncocytomas showed different immunostaining patterns for Ksp-cadherin and CD82 ($P < 0.01$, both), and mutant *FLCN*-associated HOCTs and sporadic chRCCs showed different immunostaining patterns for CK7 ($P < 0.01$) using Chi square test.

(–), < 5% of cells positive; (+), 5 to 10% positive; (++), > 10% positive; ccRCC, clear cell renal cell carcinoma; chRCC, chromophobe renal cell carcinoma; HOCT, hybrid oncocytic/chromophobe tumor; NE, not evaluated.

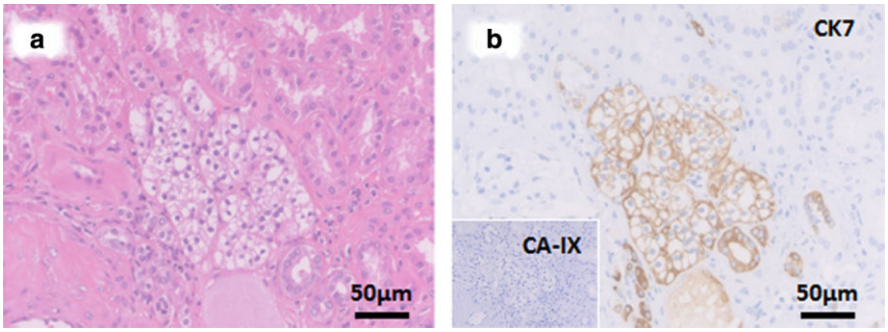

Figure 3 Histological findings of an oncocytic cell nest of a Birt-Hogg-Dubé syndrome patient's kidney. (**a**) Hematoxylin and eosin staining of an epithelial cell nest localized in the normal-looking renal cortex of P4-T1. (**b**) Immunostaining for CK7 in a serial section of (a). The nest cells are positively stained for CK7. Inset: The cells of the same lesion are negatively stained for CA-IX.

CK7. All 10 clear cell RCCs failed to stain for CK7. All oncocytomas were diffusely stained for S100A1 (100%), but nine of 11 chromophobe RCCs were either negative or sparsely stained for S100A1. All clear cell RCCs, but not other tumor types, were positively stained for CA-IX (100%). Statistical analysis revealed that mutant *FLCN*-associated HOCTs and sporadic oncocytomas showed different immunostaining patterns for Ksp-cadherin and CD82 ($P < 0.01$, both), and that mutant *FLCN*-associated HOCTs and sporadic chromophobe RCCs showed different immunostaining patterns for CK7 ($P < 0.01$).

Immunohistochemical findings of oncocytic cell nests

Normal-looking cortices in the vicinity of mutant *FLCN*-associated tumors often contained oncocytic cell nests and a few atypical tubules (Fig. 3a). It has been suggested that these lesions are the pre-neoplastic buds of multiple tumors in BHD. Immunostaining patterns of these lesions were examined in seven cases. In all seven lesions, the cell nests were positively stained for CK7 (Fig. 3b), and were also weakly stained for Ksp-cadherin, but were not stained for CA-IX, CD82, or S100A1.

The normal-looking tubules and glomeruli of BHD kidneys showed the same immunostaining patterns as normal kidneys.

DISCUSSION

In most cases, mutant *FLCN*-associated renal tumors are composed of granular oncocytic cells and clear cells. HOCTs, regardless of genetic background, are currently placed within the chromophobe RCC category.[14] Mutant *FLCN*-associated HOCTs show an admixture of areas typical of oncocytoma and chromophobe RCC with large eosinophilic cells with intracytoplasmic vacuoles.[14] In the present study, the majority of tumors were classified into either chromophobe RCC (46.9 %) or HOCT (43.8 %). We found that most mutant *FLCN*-associated chromophobe RCCs and HOCTs were S100A1$^+$, Ksp-cadherin$^+$, CD82$^+$ and CA-IX$^-$. The positive staining for Ksp-cadherin and CD82 was similar to the staining pattern of sporadic chromophobe RCCs. The majority of oncocytic mutant *FLCN*-associated tumors and sporadic oncocytomas shared diffuse staining for S100A1. The HOCTs and chromophobe RCCs often occurred simultaneously in the affected kidney of a patient with BHD. Since only five markers were examined, we cannot exclude the possibility that HOCTs and chromophobe RCCs might demonstrate differential patterns for other antibodies. The current study suggested immunohistochemical similarities between HOCTs and chromophobe RCCs. In addition, the following features in mutant *FLCN*-associated HOCTs were demonstrated. Oncocytoma-looking cells potentially demonstrated chromophobe RCC-like immunoreactivities, and chromophobe RCC-looking cells frequently had oncocytoma-like immunoreactivities. Both tumor types in this study were clinically stage I at the time of surgical intervention, and patients were alive without metastasis. Possible differences in biological aggressiveness need to be further studied in a larger number of cases.

Mutant *FLCN*-associated renal tumors frequently have foci of clear cell-looking cytoplasm.[6,9] Not only the cells with water-clear cytoplasm but also the cells with prominent perinuclear halo might give an impression of clear cell RCC. The immunohistochemical study of our cases revealed that clear cytoplasm in chromophobe RCCs and HOCTs was frequently immunostained for Ksp-cadherin, CK7 and CD82, but not for CA-IX. The results indicate that clear cell-looking foci in chromophobe RCCs and HOCTs are probably derived from distal tubules or collecting ducts. The remaining three tumors diagnosed as clear cell RCC demonstrated a distinctively different immunostaining pattern, suggesting that the affected cells were derived from proximal tubules.

Oncocytic cell nests in normal-looking renal cortices might be pre-neoplastic buds of multiple RCCs in BHD. Positive immunoreactivity of these nests for CK7 and Ksp-cadherin indicated that they derived from distal tubules or collecting ducts. The results support the hypothesis that

these epithelial nests in patients with BHD developed into chromophobe RCC or HOCT. With regard to clear cell RCC, further study is needed to clarify pathological signs of pre-cancerous events in proximal tubules. It should be carefully determined whether and how these epithelial nests are associated with various histological types of mutant *FLCN*-associated tumors. Most of the examined tumors were macroscopically located in the cortex. Since significant numbers of BHD cases with various histological types have been reported, it is likely that more than one renal cell type in the cortex is prone to carcinogenesis under the influence of haploinsufficient *FLCN*.

The present study described immunohistochemical features of mutant *FLCN*-associated renal tumors. Further study is required to clarify pathological characteristics and biological behaviors of these tumors. Future studies should also investigate whether mutant *FLCN*-associated renal tumors have genetic characteristics that are similar to their sporadic counterparts. Better understanding of the tumor pathobiology will contribute to developing appropriate therapy for BHD patients bearing multiple renal tumors.

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DISCLOSURE

We have no conflict of interest.

REFERENCES

1 Linehan WM, Srinivasan R, Schmidt LS. The genetic basis of kidney cancer: A metabolic disease. *Nat Rev Urol* 2010; **7**: 277–85.
2 Birt AR, Hogg GR, Dube WJ. Hereditary multiple fibrofolliculomas with trichodiscomas and acrochordons. *Arch Dermatol* 1977; **113**: 1674–7.
3 Hornstein OP, Knickenberg M. Perifollicular fibromatosis cutis with polyps of the colon–a cutaneo-intestinal syndrome sui generis. *Arch Dermatol Res* 1975; **253**: 161–75.
4 Nickerson ML, Warren MB, Toro JR *et al*. Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt-Hogg-Dube syndrome. *Cancer Cell* 2002; **2**: 157–64.
5 Adley BP, Smith ND, Nayar R, Yang XJ. Birt-Hogg-Dube syndrome: Clinicopathologic findings and genetic alterations. *Arch Pathol Lab Med* 2006; **130**: 1865–70.
6 Pavlovich CP, Walther MM, Eyler RA *et al*. Renal tumors in the Birt-Hogg-Dube syndrome. *Am J Surg Pathol* 2002; **26**: 1542–52.
7 Hasumi H, Baba M, Hasumi Y *et al*. Regulation of mitochondrial oxidative metabolism by tumor suppressor FLCN. *J Natl Cancer Inst* 2012; **104**: 1750–64.
8 Klomp JA, Petillo D, Niemi NM *et al*. Birt-Hogg-Dube renal tumors are genetically distinct from other renal neoplasias and are associated with up-regulation of mitochondrial gene expression. *BMC Med Genomics* 2010; **3**: 59.
9 Houweling AC, Gijezen LM, Jonker MA *et al*. Renal cancer and pneumothorax risk in Birt-Hogg-Dube syndrome; an analysis of 115 FLCN mutation carriers from 35 BHD families. *Br J Cancer* 2011; **105**: 1912–9.
10 Kuroda N, Furuya M, Nagashima Y *et al*. Intratumoral peripheral small papillary tufts: A diagnostic clue of renal tumors associated with Birt-Hogg-Dube syndrome. *Ann Diagn Pathol* 2014; **18**: 171–6.
11 Furuya M, Tanaka R, Koga S *et al*. Pulmonary Cysts of Birt-Hogg-Dube Syndrome: A Clinicopathologic and Immunohistochemical Study of 9 Families. *Am J Surg Pathol* 2012; **36**: 589–600.
12 Truong LD, Shen SS. Immunohistochemical diagnosis of renal neoplasms. *Arch Pathol Lab Med* 2011; **135**: 92–109.
13 Ohe C, Kuroda N, Takasu K *et al*. Utility of immunohistochemical analysis of KAI1, epithelial-specific antigen, and epithelial-related antigen for distinction of chromophobe renal cell carcinoma, an eosinophilic variant from renal oncocytoma. *Med Mol Morphol* 2012; **45**: 98–104.
14 Srigley JR, Delahunt B, Eble JN *et al*. The International Society of Urological Pathology (ISUP) Vancouver Classification of Renal Neoplasia. *Am J Surg Pathol* 2013; **37**: 1469–89.