Clinicopathological and molecular features of hereditary leiomyomatosis and renal cell cancer-associated renal cell carcinomas

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ABSTRACT

Aims Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant disorder caused by germline mutations in fumarate hydratase (FH). Affected families have an increased risk of renal cell carcinoma (RCC). HLRCC-associated RCC (HLRCC-RCC) is highly aggressive. Clinicopathological information of genetically diagnosed patients with HLRCC-RCC contributes to the establishment of effective therapies.

Methods Ten Japanese patients with HLRCC-RCC were enrolled in the study. Genetic testing for *FH* was carried out. Somatic mutations in *FH* and immunohistochemical analyses of FH and B7 family ligands (PD-L1 and B7-H3) were investigated in 13 tumours. Copy number variations were evaluated in two tumours.

Results All patients had *FH* germline mutations. Regarding histology, most tumours had type 2 papillary architecture or tubulocystic pattern or both. All tumours were FH deficient by immunohistochemistry. Ten tumours were positive for PD-L1, and 12 tumours were positive for B7-H3. Somatic mutation analysis demonstrated loss of heterozygosity of *FH* in 10 tumours. Copy number variation analysis revealed uniparental disomy between 1q24.2 and 1q44 encompassing *FH*; gain of chromosome 2 p was also common. All patients had either metastases or residual tumours. Three patients died of HLRCC-RCC and one of colon cancer, whereas the other six are currently alive, including two without recurrence.

Conclusions HLRCC-RCCs appear to have unique molecular profiles, including PD-L1 expression. One patient had complete response to immunotherapy, which may be an option for HLRCC-RCC.

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INTRODUCTION

Hereditary leiomyomatosis and renal cell cancer (HLRCC; OMIM #150800), also called Reed's syndrome, is an inherited disorder characterised by leiomyomatosis of the skin and uterus, as well as renal cell carcinoma (RCC).[1 2] The responsible gene, fumarate hydratase (*FH*), is located on chromosome 1q43[2]; FH is an enzyme involved in the tricarboxylic acid cycle and plays an important role in the production of ATP.[3] Diagnoses of HLRCC by genetic testing are increasing. Most women with HLRCC have uterine leiomyomas; however, uterine leiomyoma is also common among adult women without genetic disorders. Skin papules and nodules do not necessarily occur in all mutation carriers.[4] The rate of RCC morbidity in HLRCC families is estimated to be ~15%,[5 6] and multifocal carcinogenesis is rare.[7] Therefore, physicians have difficulty recognising the risk of HLRCC in affected patients without a conspicuous medical history.

HLRCC-associated RCC (HLRCC-RCC) is defined as a distinct entity in the classification of renal tumours by WHO. HLRCC-RCC is typically aggressive and metastatic, and thus a correct diagnosis is important to perform multidisciplinary therapy.[8] A series of histological analyses of HLRCC-RCC revealed various morphologies, including type 2 papillary, solid, sieve-like and tubulocystic patterns.[9–11] According to genome studies by The Cancer Genome Atlas Research Network, HLRCC-RCC with a papillary architecture is included among cancers with the CpG island methylator phenotype,[12] which comprise not only HLRCC-RCC but also sporadic *FH*-mutated RCC.[10 12] Chromosomal events in HLRCC-RCC in relation to *FH* germline mutations are not fully understood.

In the present study, we investigated 13 RCCs from 10 HLRCC Japanese individuals with *FH* germline mutations. We performed somatic *FH* mutation analysis, immunohistochemical analysis of FH and B7 family ligands in 13 tumours. In addition, we performed copy number variation (CNV) analysis in two tumours.

MATERIALS AND METHODS

Case selection

Ten adult patients with RCCs who underwent either partial or radical nephrectomy at our institutes and were suspected of HLRCC-RCCs according to histological analyses and/or family history were included in this study. We provided genetic counselling before and after genetic testing to all but two patients who were deceased prior to the study. Written informed consent was obtained from each

patient. Resected tissues were fixed in 10% buffered formalin and embedded in paraffin. H&E staining was performed for histological diagnosis. Genetic testing was performed in the patients with high grade RCC who met at least one of the following previously proposed criteria[6 13]: (1) cutaneous leiomyoma, (2) uterine leiomyoma, (3) FH-deficient RCC according to immunohistochemistry and (4) familial history of cutaneous leiomyoma and/or FH-deficient RCC. The patients were also evaluated for diseases other than RCC. The histological type of RCC was determined by two pathologists with expertise in FH-deficient RCC (YN and CO).

Immunohistochemistry

Immunohistochemical analysis of FH and B7 family proteins was done to determine the expression of these proteins in tumour specimens. The B7 family proteins investigated were PD-L1 (programmed death-ligand 1, also known as B7 homolog 1) and B7-H3. Immunohistochemical analysis of FH and PD-L1 was performed using an automated staining platform (BenchMark ULTRA; Roche, Mannheim, Germany), according to the manufacturer's protocol, and that B7-H3 was performed using ENVISION kits (Agilent, Santa Clara, California, USA). Paraffin sections (4 µm thick) were autoclaved at 121°C for 15 min retrieval solution provided by Agilent. The working dilutions were 1:500 for the anti-FH antibody (ab95950, Abcam, Cambridge, UK), 1:200 for the anti-B7-H3 antibody (D9M2L, Cell Signaling Technology, Danvers, Massachusetts, USA), and 1:100 for the anti-PD-L1 (E1L3N, Cell Signaling Technology) antibody. The immunostaining pattern of PD-L1 in tumour cells was classified according to the algorithm edited by International Association for the Study of Lung Cancer as follows: membranous immunoreactivity at least intermediate intensity was considered 'positive'; 0, no staining or <1% membrane staining; 1+, 1%–49% membrane staining and 2+, ≥50% membrane staining. The immunostaining patterns of FH and B7-H3 in tumour cells were scored as follows: 0, no staining or positive staining in <5% cells; 1+, positive staining in 5%–10% cells; and 2+, positive staining in ≥10% cells.

DNA isolation

DNA from peripheral blood leukocytes was obtained using the LabPass Blood Mini kit (Cosmo GENETECH, Seoul, Korea). Blood samples were not available from patients 1 and 7 because they died before the analysis; their DNA was extracted from normal renal tissues using the QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. The tumour DNA of patients 1 and 2 was obtained from snap frozen tissues, and those of the other patients were obtained from macro-dissected tissues fixed in formalin and embedded in paraffin (FFPE) using the QIAamp DNA Mini kit (Qiagen).

Direct sequencing

For direct sequencing analyses, exons 1–10 of the *FH* gene were amplified by PCR using previously described primers.[5 14] The PCR conditions were as follows: 95°C for 5 min, 35 cycles at 96°C for 5 s, 60°C for 5 s and 68°C for 3 s, followed by an extension step at 72°C for 1 min after the last cycle. After purification, DNA was labelled using the Big Dye Terminator V.3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Tokyo, Japan) and sequenced using the Applied Biosystems Incorporation Prism 3100 Genetic Analyzer (Thermo Fisher Scientific).

Somatic FH mutation analysis in resected tumors

The tumour DNA of each patient was used for *FH* mutation analysis. If the mutation sequence was amplified only and the wild-type sequence undetected, or vice versa, in the direct sequencing analyses, it was determined that loss of heterozygosity (LOH) had occurred as the second hit.[15] Exons other than genetically mutated sites were also amplified, and the PCR products were sequenced to identify small intragenic mutations as potential second hits.

Table 1 The clinical features and family history of hereditary leiomyomatosis and renal cell cancer-associated manifestations in the 10 study patients

Patient (ref. no)	Age*	Sex	CLM (age)	ULM (age)	Other diseases (age)	Family history
1[17]	32	M	No	Not applicable	No	Lung ca. (father)
2[18]	34	F	No	Yes (28)	No	ULM (mother, sister) RCC (sister)
3[19]	25*	M	Yes (33)	Not applicable	No	CLM (father) ULM (two aunts, two cousins)
4	34	M	Yes (40)	Not applicable	No	ULM (two sisters) RCC (father)
5	34	F	No	Yes	No	No
6	49	M	No	Not applicable	Diabetes	ULM (mother)
7	59*	M	No	Not applicable	Dialysis (60) colon ca. (77)	ULM (mother, sister)
8	50	M	No	Not applicable	No	Breast ca. (mother)
9	44	M	No	Not applicable	No	ULM and CLM (mother), RCC (mother, uncle)
10	58*	M	Yes (39)	Not applicable	No	Intestinal ca. (brother)

*Age at initial therapy in bilateral RCC patients.
ca, carcinoma; CLM, cutaneous leiomyoma; RCC, renal cell carcinoma; ULM, uterine leiomyoma.

CNV and LOH analyses using the CytoScan HD Array

The tumour DNA of patients 1 and 2 obtained from snap frozen tissues were used for CytoScan high-density (HD) Array (Affymetrix, Santa Clara, California, USA). The resulting data were analysed using Chromosome Analysis Suite, V.4 (Affymetrix) to detect CNVs and LOH. The assessments of CNV and LOH were performed according to the manufacturer's protocol, as described previously.[16]

RESULTS

Clinicopathological information

Ten Japanese patients with HLRCC-RCC (two women, eight men) were enrolled in the study. The median age at which they underwent initial nephrectomy (n=8) or neoadjuvant therapy prior to nephrectomy (n=2) was 45 (range 25–59) years. The clinical features of the 10 probands are summarised in table 1; those of patients 1–3 were reported previously.[17–19] Three patients developed bilateral RCCs: Patients 3 and 10 developed synchronous RCCs, and patient seven developed metachronous RCCs at ages 59 and 73 years. Cutaneous leiomyomas were histologically diagnosed in three patients. Two female patients had uterine leiomyomas.

Four family members of the 10 probands had RCC. The mother of patient 9, who was diagnosed with HLRCC by genetic testing, had clear cell RCC (data not shown). Another three

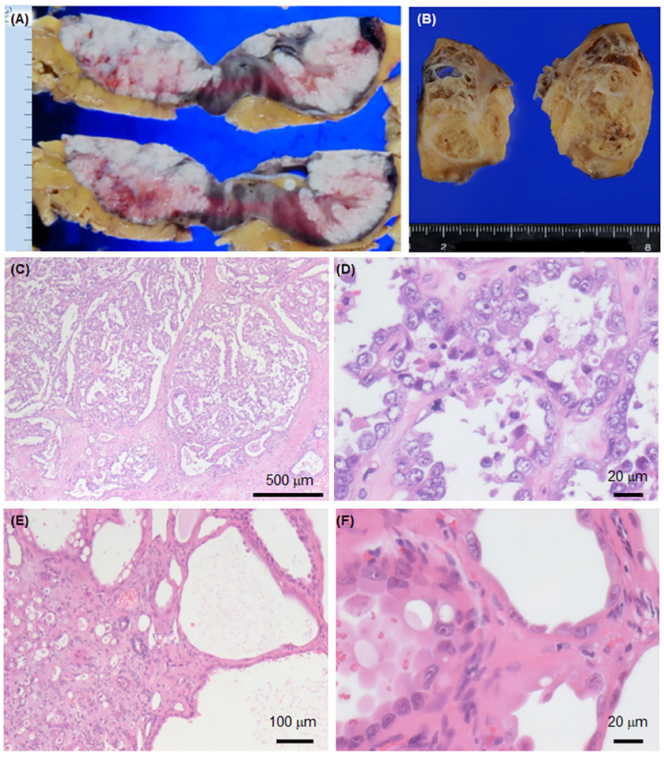

Figure 1 Macroscopic and microscopic features of hereditary leiomyomatosis and renal cell cancer (HLRCC)-associated-renal cell carcinoma (RCC). (A) Surface section of an RCC specimen from patient 6. The kidney parenchyma was highly infiltrated with white confluent masses. (B) Surface section of an RCC specimen from patient 7. A multicystic lesion with ill-defined borders was observed. (C, D) Type two papillary RCC from patient six at low (C) and high (D) magnifications. Pleomorphic nuclei and mitotic activities were noted. (E, F) Tubulocystic RCC pattern from patient 7 at low (E) and high (F) magnifications. Nuclear pleomorphism was subtle, but the tumour cells harboured eosinophilic nucleoli with halos.

untested relatives died of RCC: the sister of patient 2 at age 34 years, the father of Patient 4 at age 33 years, and the uncle of patient 9 at age 68 years. The HLRCC-associated manifestations of the relatives are summarised in table 1. HLRCC had not been suspected in the relatives who died of RCC until their probands were diagnosed by genetic testing.

All patients had advanced RCCs (figure 1A,B). Four patients died: patients 1, 2 and 4 of RCC and patient 7 of colon cancer. The other patients are alive receiving multidisciplinary therapy; among them, patients 3 and 6 had multifocal metastases that were completely removed by metastasectomy or immunotherapy, and they are disease free at present. Histologically, six tumours had type 2 papillary architecture (figure 1C,D), three of which coexisted with tubulocystic area. The other tumours demonstrated tubulocystic pattern (figure 1E,F), mucinous tubular and spindle cell carcinoma-like pattern (n=2), and a low-grade oncocytic feature reminiscent of succinate dehydrogenase (SDH)-deficient RCCs (n=2) (online supplementary figure). All resected tumours had, at least in part, characteristic nuclei harbouring enlarged eosinophilic nucleoli with a halo. The tumour size, stage, grade and clinical course are summarised in table 2.

Pathogenic germline and somatic mutations in FH

Ten probands and nine relatives were confirmed to have germline *FH* mutations by genetic testing. None of the probands shared identical mutations, and thus each was considered to belong to an unrelated family lineage. Six families had a missense mutation, three had a frameshift mutation, and one had a splice-site mutation. Somatic mutations in *FH* within the tumours were also examined. The DNA isolated from the FFPE tissues of patient 3 failed to amplify by PCR. The other tumours showed an LOH pattern, in that the wild-type allele was almost completely replaced by a mutated *FH* allele in nine tumours and vice versa in one tumour (left tumour of patient 10). The tumour of patient 8 exhibited LOH in exon 1 of *FH* and an additional second-hit intragenic mutation in exon 5. The pathogenic germline and somatic second-hit mutations in *FH* are shown in table 3.

Immunostaining of FH and B7 family ligands

We performed immunostaining of the FH and B7 family ligands (PD-L1 and B7-H3) in 13 tumours from 10 patients, including one biopsied specimen from patient 9, who received neoadjuvant therapy with sunitinib for 3 months after biopsy and then underwent radical nephrectomy. The left kidney of patient 7, which was resected 21 years prior to the current study, was not available for immunohistochemical analysis.

In non-tumour renal areas of the HLRCC patients (n=10), significant staining of FH was observed in the cytoplasm of the proximal and distal tubules. On the other hand, none of the HLRCC-RCCs showed FH staining (figure 2A,B), suggesting FH deficiency at the protein level in tumour cells.

Next, we examined the expression of B7 family ligands on tumour cell membranes. Seven tumours demonstrated 2+immunostaining of PD-L1 (figure 2C). Three tumours including one biopsied specimen showed 1+immunostaining of PD-L1. The remaining tumours including two oncocytic tumours showed negative staining. In the tumours with an area of tubulocystic carcinoma, cystic glands were scarcely stained for PD-L1. In patient 9, who underwent neoadjuvant therapy with sunitinib for 3 months, the biopsied specimen prior to neoadjuvant therapy showed scattered immune cells, whereas the resected tumour after neoadjuvant therapy was significantly infiltrated with lymphocytes (figure 2D). In this case, the resected tumour showed markedly more PD-L1-positive cells compared with the biopsied specimen (figure 2E). In the B7-H3 immunohistochemical analyses, all but one tumour showed positive staining; seven tumours were focally positive and five diffusely positive (figure 2F). The results of the immunohistochemical analyses are summarised in table 3.

Broad chromosomal analysis of CNVs

The DNA obtained from snap frozen tumour tissues (ie, those from patients 1 and 2) was used for CNV analysis. Patient 1 had gains in chromosomes 2, 7, 16 and 17, and patient 2 had gains in chromosomes 2 p and 3q and losses in chromosomes 9q, 13 and 18. Copy-neutral LOH, namely uniparental disomy (UPD), in 1q and a gain in 2 p were observed in both tumours. Other UPD regions included chromosome 18 in patient 1 and 5q31.1–5q35.3 in patient 2. The copy number ratios and allele peaks are shown in figure 3.

Both tumours had common LOH regions across a wide range of chromosomes (figure 4A). The largest of these regions was between 1q24.2 and 1q44, encompassing *FH* at 1q43. Short segmental analysis of regions proximal to *FH* confirmed the UPD pattern (figure 4B). Among the other segmental regions of LOH shared between patients 1 and 2, there were 14 cases of UPD. The chromosomal regions and genes showing LOH in both patients 1 and 2 are listed in online supplementary table 1.

Table 2 Tumor size, stage, histology, grade and clinical course of the 10 study patients

Patient (ref. no)	Size (cm)	Stage (TNM)	Histology	ISUP grade	Surgery	Metastases (residual)	Therapy	Prognosis (mo)
1[17]	10×8	III (pT3aN0M0)	Type two pap and TC	3	Radical	Liver	Gemcitabine, nedaplatin, arterial embolization, tegafur, uracil	DOD (17)
2[18]	6.8×5.5	III (pT3aN0M0)	Type two pap and TC	3	Radical	Bone, lung	Sunitinib, axitinib, nivolumab, everolimus, temsirolimus	DOD (35)
3[19]	Lt: 10×9.5 Rt: 2.5×2.5	Lt: IV (pT2aN0M1) Rt: I (pT1aN0M0)	Lt: MTSC Rt: oncocytic	Lt: 3 Rt: 2	Lt: radical Rt: partial	Liver, lung	Hepatectomy lung lobectomy	NED (123)
4	9×8	II (pT2aN0M0)	Type two pap	4	Radical	Bone	Sunitinib, axitinib, temsirolimus, everolimus, nivolumab, pazopanib, radiation	DOD (79)
5	5×4.3	IV (pT1bN1M1)	Type two pap and TC	3	Radical	Lymph node	Axitinib	AWD (20)
6	10×6	III (pT3aN0M0)	Type two pap	3	Radical	Peritoneal cavity	Ipilimumab, nivolumab	NED (19)
7	Lt: 4×3 Rt: 4.5×3.5	Lt: I (pT1aNxM0) Rt: III (pT3aNxM0)	Lt: TC Rt: TC	Lt: 2 Rt: 3	Lt: radical Rt: partial	Liver, lung	Partial hepatectomy interferon	DEAD (144)
8	7	T		4	Radical	Bone, lung, lymph node	Radiation, sunitinib, nivolumab	AWD (42)
9	6×5	IV (cT4N0M1)	TC	4	Radical	Bone, lung, liver	Sunitinib, nivolumab, axitinib	AWD (19)
10	Lt: 1.7×1.7 Rt: 1.7×1.5	Lt: I (pT1aN0M0) III (pT3aN0M0)	Lt: MTSC Rt: TC and oncocytic	Lt: 3 Rt: 2	Lt: partial Rt: partial	Residual tumour (Lt)	Watchful surveillance	AWD (20)

AWD, alive with the disease; DEAD, died of diseases other than renal cell carcinoma; DOD, died of the disease (renal cell carcinoma); ISUP, international society of urological pathology; Lt, left; mo, month; MTSC, mucinous tubular and spindle cell carcinoma-like pattern; NED, no evidence of the disease; pap, papillary pattern; Rt, right; TC, tubulocystic pattern; TNM, tumor (T), nodes (N), and metastases (M).

The causative genes of hereditary RCCs include *VHL* and *MET*. Although patient 1 had a gain in *MET* and patient 2 had a loss in *VHL*, the events were not shared (figure 4B). Among other genes associated with sporadic RCCs, the chromatin-remodelling genes *BAP1* and *PBRM1* showed UPD in one tumour but had no copy number alterations in the other tumour. Another chromatin-remodelling gene, *SETD2* and the tumour suppressor genes *CDKN2A* and *CDKN2B* had neither copy number alterations nor LOH in these tumours (data not shown). The chromosomal regions and genes showing losses or gains that were present in both tumours are listed in online supplementary tables 2 and 3, respectively.

DISCUSSION

In the present study, sequence analysis showed that all tumours had LOH of *FH*. In addition, CNV analysis of two cases demonstrated that UPD comprised a great part of chromosome 1q, in which *FH* is located. A previous next-generation sequencing study of FH-deficient tubulocystic RCCs (n=9) showed either homozygous or heterozygous *FH* mutations in eight cases and copy number loss one case.[10] Together with the present study, the results suggest that LOH of *FH* is a frequent event in the majority of HLRCC-RCCs, whereas copy number loss is not necessary. Limitations of this study include that only two cases were available for CNV analysis. It should be noted that LOH of *FH* alone may not indicate the aggressiveness of HLRCC-RCC, because it is also observed in benign leiomyomas in HLRCC patients.[19–21] Since numerous genes between chromosomal regions 1q24.2 and 1q44 showed UPD in our study, some of those adjacent to *FH* may contribute to the aggressive behaviour of HLRCC-RCC. A previous array comparative genomic hybridisation study of 11 HLRCC-RCCs identified gains in chromosome 2 in four tumours and losses in chromosome 18 in three tumors.[22] In our study, HLRCC-RCCs in patients 1 and

Table 3 Immunohistochemical analyses and germline and somatic *FH* mutations in cancer cells

	Immunohistochemistry			FH mutations	
		B7 family ligands			
Patient (ref. no)	FH	PD-L1	B7-H3	Germline *FH* mutation (protein level)	Somatic *FH* mutation
1[17]	0	1+	1+	Exon 2c.251_267+7del (Exon 2 skip)	Exon 2 LOH
2[18]	0	2+	2+	Exon5c.675del (p.Phe227LeufsX44)	Exon 5 LOH
3[19]	Lt: 0 Rt: 0	Lt: 2+ Rt: 0	Lt: 2+ Rt: 0	Intron3c.379-2A>G (Exon 4 skip)	Lt: undetectable Rt: undetectable
4	0	1+	2+	Exon8c.1229C>T (p.Pro410Leu)	Exon 8 LOH
5	0	2+	1+	Exon5c.566A>G (p.Asp189Gly)	Exon 5 LOH
6	0	2+	2+	Exon5c.641_642del (p.Leu214SerfsX1)	Exon 5 LOH
7	Lt: ND Rt: 0	Lt: ND Rt: 0	Lt: ND Rt: 1+	Exon7c.1067T>C (p.Leu356Ser)	Lt: Not done Rt: Exon 7 LOH
8	0*	2+*	1+*	Exon1c.77C>T (p.Pro26Leu)	Exon 1 LOH, Exon 5c.703C>T
9	Biopsy: 0 Resect: 0*	Biopsy: 1+ Resect:2+*	Biopsy: 1+ Resect: 1+*	Exon7c.1002T>G (p.Ser334Arg)	Exon 7 LOH
10	Lt: 0 Rt: 0	Lt: 2+ Rt: 0	Lt: 2+ Rt: 1+	Exon4c.431G>C (p.Gly144Ala)	Lt: Exon 4 LOH† Rt: Exon 4 LOH

*Status after neoadjuvant therapy.
†LOH involving the wild-type allele.
FH, fumarate hydratase; LOH, loss of heterozygosity involving the mutant allele; Lt, left; ND, not done; Rt, right.

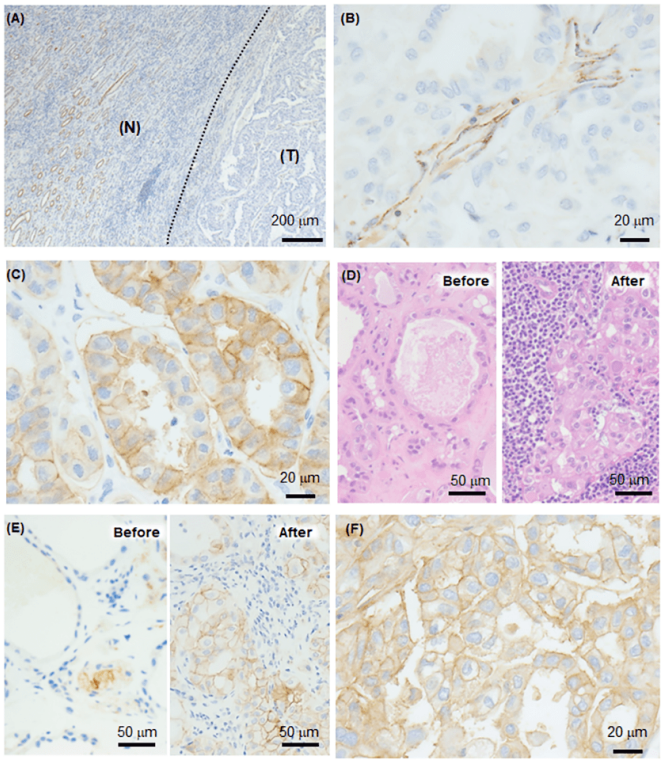

Figure 2 Fumarate hydratase (FH) and B7 family protein expression in hereditary leiomyomatosis and renal cell cancer (HLRCC)-associated-renal cell carcinoma (RCC). (A) Immunostaining of FH in the tumour border region of patient 5 at low magnification. FH was strongly positive in normal-looking tubules, whereas it was negative in tumour cells. (N) and (T) indicate normal and tumour areas, respectively. (B) Immunostaining of FH in the RCC of patient 2 at high magnification. Tumour cells were negative, whereas capillary endothelial cells were positive, for FH. (C) Immunostaining of PD-L1 in the RCC of patient 2. Tumour cells had strong membranous staining. (D) H&E staining of the tumour from patient 9 before (left) and after (right) neoadjuvant therapy. The latter was highly infiltrated with lymphocytes. (E) Immunostaining of PD-L1 in the RCCs of patient 9. PD-L1-positive cells were sparse (left) in the biopsied specimen before neoadjuvant therapy but diffusely distributed in the resected tumour after neoadjuvant therapy. (F) Immunostaining of B7-H3 in the RCC of patient 2. Tumour cells showed strong membranous staining.

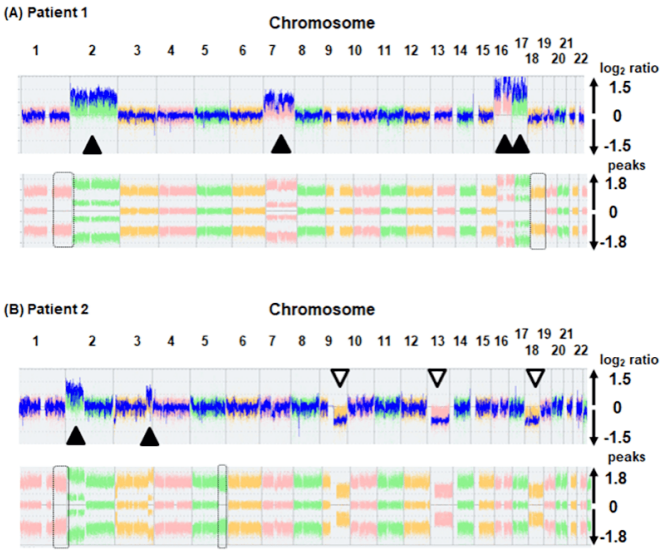

Figure 3 Copy number ratios and allele peaks. Copy number ratios [$\log_2$ ratios (upper panels)] and allele peaks (lower panels) for all chromosomes (left end, chromosome 1; right end, chromosome 22) in the tumours of patient 1 (A) and patient 2 (B). (A) Chromosomes 2, 7, 16 and 17 showed increased $\log_2$ ratios (black arrowheads). The lower panels corresponding to each of these chromosomes show tetramodal allele peaks, indicating trisomy. Chromosomes 1q and 18 demonstrated a normal $\log_2$ ratio but bimodal allele peaks (dotted rectangles in the lower panel), indicating uniparental disomy. (B) Chromosomes 2 p and 3q26.2–29 showed increased $\log_2$ ratio (black arrowheads) and chromosomes 9q, 13 and 18 decreased $\log_2$ ratios (white arrowheads). The lower panels corresponding to each of these chromosomes show either tetramodal (2 p and 3q26.2–29) or narrow bimodal allele peaks (9q, 13 and 18), indicating copy number alterations. chromosomes 1q and 5q31.1–35.3 demonstrated a normal $\log_2$ ratio but bimodal allele peaks (dotted rectangles in the below lower panel), indicating uniparental disomy.

2 had a 2 p gain, and patient 1 had UPD and patient 2 had a loss in chromosome 18. Koski *et al* showed a specific loss in the *CDH19* gene at 18q22.1.[22] In the present cases, either UPD or copy number loss in *CDH19* was noted, but the events were not restricted to *CDH19*, rather covering all of chromosome 18. Potential key genes in these chromosomal regions and their roles in HLRCC-RCC should be clarified in the future using a larger number of cases.

The clinical trial involving patients with treatment-naïve advanced or metastatic clear cell RCC demonstrated that nivolumab plus ipilimumab increased response rates compared with sunitinib alone.[23] Some patients with advanced papillary RCC had a significant therapeutic response to nivolumab.[24 25] On the other hand, evidence-based therapeutic strategies for HLRCC-RCC have not been established. Although immune checkpoint blockade is expected to improve the survival of patients with a poor outcome metastatic RCCs, the information on immunotherapy for HLRCC-RCC is sparse. In a previous study by Alaghehbandan *et al* using clone E1L3N, 9 of 13 FH-deficient RCCs showed various positive immunostaining of PD-L1.[26] We also used E1L3N for PD-L1 analysis because it shows good concordant with some commercial assays.[27 28] Our immunohistochemical results supported those findings. Patients 6 with PD-L1-positive RCC received nivolumab in combination with ipilimumab, which resulted in a complete response. The RCC of patient 9 displayed increased expression of PD-L1 and lymphocytic infiltration after neoadjuvant therapy. However, adjuvant nivolumab failed to reduce the sizes of metastases; axitinib was then administrated and stable disease was achieved. Another B7 family ligand, B7-H3, is also a potential target of cancer immunotherapy.[29] We found that B7-H3 was expressed on the tumour cell surface in all but one case. Since B7-H3 is rarely expressed in normal renal tissues, blockade of B7-H3 function may augment anti-tumour immunity with minimal side effects.[29] If a large number of effector T lymphocytes are successfully recruited to the tumours, PD-L1- and/or B7-H3-positive tumour cells are expected to respond to immunotherapy.[30 31]

Histological studies have revealed similar morphologies between HLRCC-RCCs and some other renal tumours such as collecting duct carcinomas, tubulocystic carcinomas and SDH-deficient RCCs.[10 11 32] Such histological complexity often results in the failure of pathologists to recognise the possibility of HLRCC. Smith *et al* reported that four FH-deficient RCCs reminiscent of SDH-deficient RCC had no metastatic lesions, and one case had no copy number loss/gain.[32] Two oncocytic

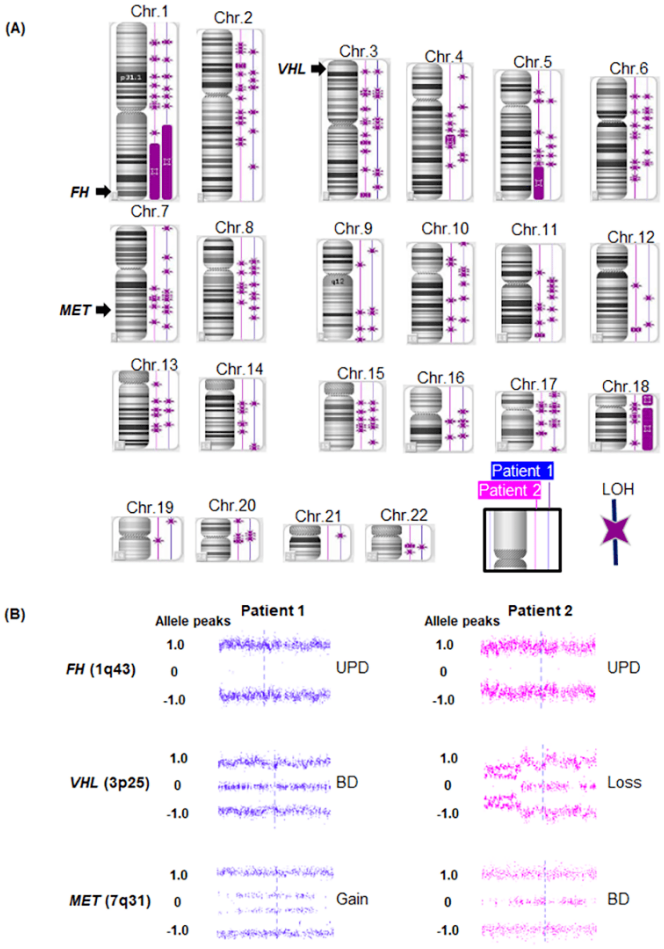

Figure 4 Loss of heterozygosity (LOH) detected by the CytoScan HD array and chromosomal regions of hereditary kidney cancer-associated genes with allele peaks. (A) Purple stars indicate LOH (right, patient 1; left, patient 2). LOH in chromosomes (Chr) 1–22 in the two tumours are shown. The chromosomal positions of three hereditary kidney cancer-associated genes (*FH*, *VHL* and *MET*) are indicated by arrows. The clinicopathological features of patients 1and 2 are presented in table 2. (B) Allele peaks in the proximal segments of *FH*, *VHL* and *MET*. The allele peaks of patients 1 (purple) and 2 (rose colour) are shown on the left and right, respectively. Bimodal, trimodal, and tetramodal allele peaks indicate uniparental disomy (UPD), biparental disomy (BD), and chromosomal gain, respectively. Focal loss of the chromosomal segment containing *VHL* was seen in patient 2 (middle right). Dotted lines indicate precise gene sites. *FH*, fumarate hydratase.

tumours in our study were smaller (2.5 cm and 1.7 cm) than their contralateral main tumours, suggesting a potential condition leading to a more aggressive phenotype. Previous studies have indicated that HLRCC-RCC is predominantly unilateral.[7 22] Three of 10 patients in our study developed bilateral RCCs, including one who developed a contralateral tumour at 14 years after development of the initial tumour. The predominance of unilateral tumours might be due in part to poorly prognostic outcomes that prevent long-term follow-up. Better understanding of the histological sequence from preneoplastic to highly malignant morphologies will help pathologists determine the risk of HLRCC-RCC in previously undiagnosed patients. The difficulty with histological diagnosis of HLRCC-RCC includes the necessity of genetic testing to confirm germline *FH* mutations. Clarification of pathogenic variants after genetic counselling and patient consent may be possible only in hospitals equipped with advanced medical facilities and genetic experts. A practicable classification system will be required to provide appropriate clinical management to patients and their families with unknown genetic susceptibility.

In the present study, most of the HLRCC-RCCs expressed PD-L1 and B7-H3. Immunotherapies targeting B7 family ligands are expected to improve the prognoses of affected patients. New approaches such as chimeric antigen receptor T therapy will also be investigated in the future. We hope that our study will help oncologists establish therapeutic strategies against HLRCC-RCCs.

Take home messages

- Histological complexity such as tubulocystic and spindle morphologies often misleads pathologists into unrecognising the possibility of hereditary leiomyomatosis and renal cell cancer (HLRCC).
- Most HLRCC-renal cell carcinoma (RCCs) have loss of heterozygosity of fumarate hydratase as somatic events.
- Uniparental disomy in 1q and gain in 2 p may characterise chromosomal state of HLRCC-RCCs.
- Most HLRCC-RCCs express PD-L1 and/or B7-H3 on tumour cell membranes, and immunotherapies are expected to improve the prognoses of affected patients.

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