Review Article

Birt–Hogg–Dubé syndrome: Clinical and molecular aspects of recently identified kidney cancer syndrome

Hisashi Hasumi,1 Masaya Baba,2 Yukiko Hasumi,3 Mitsuko Furuya4 and Masahiro Yao1

1Department of Urology, Yokohama City University School of Medicine, Yokohama, Kanagawa, 4International Research Center for Medical Sciences, Kumamoto University, Kumamoto, and Departments of 3Ophthalmology and 4Molecular Pathology, Yokohama City University School of Medicine, Yokohama, Kanagawa, Japan

Abstract: Birt–Hogg–Dubé syndrome is an autosomal dominantly inherited disease that predisposes patients to develop fibrofolliculoma, lung cysts and bilateral multifocal renal tumors, histologically hybrid oncocytic/chromophobe tumors, chromophobe renal cell carcinoma, oncocytoma, papillary renal cell carcinoma and clear cell renal cell carcinoma. The predominant forms of Birt–Hogg–Dubé syndrome-associated renal tumors, hybrid oncocytic/chromophobe tumors and chromophobe renal cell carcinoma are typically less aggressive, and a therapeutic principle for these tumors is a surgical removal with nephron-sparing. The timing of surgery is the most critical element for postoperative renal function, which is one of the important prognostic factors for Birt–Hogg–Dubé syndrome patients. The folliculin gene (FLCN) that is responsible for Birt–Hogg–Dubé syndrome was isolated as a novel tumor suppressor for kidney cancer. Recent studies using murine models for FLCN, a protein encoded by the FLCN gene, and its two binding partners, folliculin-interacting protein 1 (FNIP1) and folliculin-interacting protein 2 (FNIP2), have uncovered important roles for FLCN, FNIP1 and FNIP2 in cell metabolism, which include AMP-activated protein kinase-mediated energy sensing, Ppargc1a-driven mitochondrial oxidative phosphorylation and mTORC1-dependent cell proliferation. Birt–Hogg–Dubé syndrome is a hereditary hamartoma syndrome, which is triggered by metabolic alterations under a functional loss of FLCN/FNIP1/FNIP2 complex, a critical regulator of kidney cell proliferation rate; a mechanistic insight into the FLCN/FNIP1/FNIP2 pathway could provide us a basis for developing new therapeutics for kidney cancer.

Key words: Birt–Hogg–Dubé syndrome, folliculin, kidney cancer, mitochondria, mTORC1.

In 1977, three Canadian physicians, Birt, Hogg and Dubé, reported kindred with skin nodules that they named fibrofolliculoma.1 In 1999, the development of lung cysts and renal tumors in this kindred were reported, and this disorder was thereafter called BHD syndrome.2 In 2002, FLCN was identified as a novel tumor suppressor gene responsible for BHD syndrome, and a genetic test of FLCN is used as a diagnostic tool for BHD syndrome.3 Among the clinical triad of BHD syndrome, the management of kidney cancer is a key for the overall survival of BHD patients. Although FLCN was a totally novel protein with unknown function and no known domains at the time of discovery, recent studies using murine models have uncovered important roles for FLCN in cell metabolism.4–10 In the present review article, we describe the clinical features and management of BHD syndrome, as well as the recent progress in basic research of the FLCN pathway, mainly focusing on animal models.

Clinical features of BHD syndrome

Fibrofolliculomas

After the age of 20 years, 82–92% of BHD patients in Western countries and 20–29% of Japanese Asian patients develop fibrofolliculomas, which are white papules on their face, neck and upper torso (Fig. 1).2,11–13 There might be some difference of the skin tumor incidence between the races. Because fibrofolliculomas are histologically continuous with the...
sebaceous glands and localized to sites with many sebaceous glands, such as the peri-nasal area, it is postulated that the origin of fibrofolliculomas might be sebaceous glands rather than hair follicles. Although a clinical trial for a focal treatment using rapamycin was carried out based on the result that mTORC1 is activated under FLCN deficiency, the 6-month treatments with rapamycin were not effective for 19 cases of fibrofolliculomas. Rapamycin is a large molecule, and it is difficult to make an easily absorbed ointment. Because topical rapamycin was effective for angiofibromas of tuberous sclerosis patients after a modification of ointment, an improvement of drug delivery might increase the efficacy of topical rapamycin treatment for BHD-associated fibrofolliculomas.

Lung cysts

Pulmonary cysts are seen in 90% of BHD patients, and 24–29% of BHD patients develop recurrent pneumothorax at the average age of 36 years (Fig. 2). The number of cysts varies from 30 to 400, the sizes of lung cysts vary from a few millimeters to 2 cm, and they are predominantly seen in lower and/or peripheral lesions of lungs. The histology of BHD-associated lung cysts is distinct from that of other types of lung cysts. Cuboidal cells resembling type II pneumocytes are often observed in the innermost layers of BHD-associated lung cysts, and these cells show the activation of mTORC1 and hypoxia-inducible factor–vascular endothelial growth factor pathways.

Renal tumor and its management

Between 16 and 34% of BHD patients develop renal tumors at the average age of 50 years (Fig. 3). These tumors are bilateral multifocal with diverse histologies that include HOCT (50%), chromophobe renal cell carcinoma (34%), clear cell renal cell carcinoma (9%), oncocytoma (5%) and papillary renal cell carcinoma (2%). Multifocal renal oncocytosis, composed of cells electron-microscopically filled with mitochondria and observed in the surrounding normal kidney of BHD-associated renal tumors in 50–58% of patients, is possibly a pre-cancer lesion. Kidney surveillance is recommended at 20 years-of-age. For the kidney surveillance, computed tomography or magnetic resonance imaging is more suitable than ultrasonography, because computed tomography and magnetic resonance imaging detect renal masses of 15–20 mm with 100% detection rates, whereas ultrasonography can detect those masses with 58% detection rates. We carry out the kidney surveillance every 3 years in the same manner as we survey kidneys of VHL hereditary kidney cancer syndrome. As recommended to the sporadic form of renal cell carcinoma, a complete removal of tumor lesions by partial or radical nephrectomy is primarily indicated for the treatment of BHD-related kidney cancer. However, as with other kidney cancer syndromes, having a risk of synchronous and metachronous multiple kidney cancers through the life time, the balance of oncological radicality and postoperative renal function is one of the most critical elements for the quality of life and overall survival of BHD patients. A partial nephrectomy rather than a radical nephrectomy is to be indicated for nephron sparing as far as possible. The size of the renal tumor at surgery is therefore one of the keys to success for minimizing the number of surgeries in a lifetime, as well as postoperative renal function. Based on our experiences, we operate on BHD-associated renal tumors when the diameter of the largest tumor reaches 2 cm, which is the same concept we use to operate on VHL-associated renal tumors. If renal tumors are
occurring bilaterally, one of the kidneys is operated on at an initial surgery. After confirmations of favorable renal function remaining, as well as of enough recovery of the patient’s physical condition, we will carry out a second surgery to the other kidney. Intraoperative ultrasonography is useful for detecting impalpable buried types of tumors and small lesions undetectable with preoperative imaging studies. Intraoperative ultrasonography also clarifies the architecture of blood vessels, and the distance between tumor and urinary collecting systems. In our experience, BHD-associated renal tumors usually lack a tumor pseudocapsule, which is in contrast with VHL-associated renal tumors. In this setting, intraoperative ultrasonography serves as a useful device to define a tumor margin, which is essential for both curative resections and nephron sparing. For multifocal tumors, before resecting large or buried-type tumors, relatively small sized and palpable tumors can be removed without clamping renal vessels to minimize the renal ischemic time. The proper manual compressions onto the renal ischemic time. The proper manual compressions onto the renal ischemic time. The proper manual compressions onto

**FLCN, the gene responsible for BHD syndrome**

Through a linkage analysis of BHD syndrome, *FLCN*, which is located on 17p11.2, was identified as the gene responsible for BHD syndrome. With highly accurate sequencing methods, the detection ratio of *FLCN* germline mutation is now approaching 90%.3,18,27,43 To date, more than 100 types of mutations have been reported, which include insertion/deletion, nonsense/splice site mutation.45,46 Small numbers of missense mutations have been reported, including Arg239Cys, His255Pro, Val400Ile and Lys508Arg, although a pathogenesis of these missense mutations is not well understood.47 Insertion or deletion of a cytosine in a tract of eight cytosines in exon 11 (c.1285dupC or delC) is a mutational “hot spot” that was identified in up to 50% of BHD patients regardless of human races.3,27,40,43,48,49 The database of folliculin sequence variations is available at the site hosted by Leiden Open Variation Database (https://grenada.lumc.nl/LOVD2/shared1/home.php?select_db=FLCN or http://www.skindexdatabase.com/variants.php?action=view_unique&select_db=FLCN).

**Identification of two FLCN binding partners, FNIP1 and FNIP2**

By the proteomic approach using co-immunoprecipitants pulled down by HA-tagged FLCN protein, a 130-kDa protein with unknown function was found to bind to FLCN, and this novel protein was named FNIP1.9 The same technique was applied to HA-tagged FNIP1 protein, and FNIP1 was found to bind to AMPK, which is an important energy sensor of cells, first implying that the FLCN/FNIP1 pathway might interact with metabolic pathways.9 Through bioinformatics analysis, a novel protein of which amino acid sequences are highly homologous to those of FNIP1 was identified and named FNIP2.6,50 The amino acid sequences of FNIP1 and FNIP2 show 49% identity and 74% similarity, indicating that FNIP1 and FNIP2 have overlapping functions.6,50 FNIP1 and FNIP2 form a homodimer as well as a heterodimer, and bind to FLCN through the C-terminus of FLCN.5,50 FNIP1 and FNIP2 localize predominantly in the cytoplasm, whereas FLCN localizes in both the cytoplasm and the nucleus.6,50

**Other clinical features**

It is controversial whether colon polyps and colon cancer are associated with BHD syndrome.30,35,40 Because oncocytoma of parotid gland27,39,41–43 and thyroid cancers have been reported in BHD patients, a cervical surveillance of BHD patients might be considered.27,44

---

**Fig. 3** (a) Computed tomography scan shows BHD-associated renal tumors (arrows). (b,c) Hematoxylin-eosin stain shows the most predominant forms of BHD-associated renal tumor, HOCT. (b) Low and (c) high magnification.
Lessons from animal models

Whole-body FlnC knockout mouse model

FlnC conditional knockout mouse was established by targeting exon 7 of the FlnC gene, and a whole-body deletion of the FlnC gene from both alleles was achieved using β-actin-Cre transgenic mouse. Homozygous whole-body FlnC knockout mouse was embryonically lethal from E5.5 to E6.5 with swollen vacuoles in the visceral endoderm, suggesting that FlnC might have important roles in phagocytosis, trafficking or digestion.5 Heterozygous whole-body FlnC knockout mice, which mimic human BHD syndrome, developed kidney tumors around 1.5 years-of-age. The histology of renal tumors developed in heterozygous whole-body FlnC knockout mice was similar to that of human BHD-associated tumors, which includes HOCT, clear cell renal cell carcinoma and papillary renal cell carcinoma.51 The loss of heterozygosity of the FlnC locus was observed in these tumors, underscoring FLCN as a classical tumor suppressor with the two-hit theory.5

Kidney-specific FlnC knockout mouse model

The kidney-specific FlnC knockout mouse model using cadherin 16 (CDH16 or KSP)-Cre transgenic mouse showed enlarged polycystic kidneys, and died at 3 weeks-of-age as a result of renal failure.1053 In these kidneys, mTORC1 was activated, and rapamycin treatment partially decreased the size of polycystic kidneys and prolonged the survival term.10 Mitochondrial biogenesis was increased in these kidneys and inactivation of Pparec1α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha), which is a co-activator for mitochondrial gene transcriptions, partially decreased the size of FlnC-deficient kidneys, underscoring Pparec1α as a key enhancer for aberrant kidney cell proliferations under FlnC deficiency.8

Muscle-specific FlnC knockout mouse model

Elucidation of FlnC roles in metabolic organs, such as skeletal and cardiac muscle, gave us conclusive evidence that the FlnC pathway is associated with metabolism. The muscle-specific FlnC knockout mouse model using creatine kinase, muscle (CKM)-Cre transgenic mouse showed red-colored muscle in which mitochondrial oxidative metabolism was upregulated, as well as cardiac hypertrophy in which mTORC1 and its downstream signaling molecules were activated. The muscle color and upregulated mitochondrial biogenesis of muscle-specific FlnC knockout mouse was completely reversed and cardiac hypertrophy was partially ameliorated by inactivation of Pparec1α.5,8

Whole-body Fnip1 and/or Fnip2 knockout mouse models

The Fnip1 knockout mouse showed a B cell developmental defect,54,55 red-colored muscle and cardiac hypertrophy,7,56 suggesting that FlnC and Fnip1 function co-operatively, and the deficiency of FlnC and Fnip1 might phenocopy each other. However, Fnip1 knockout mouse developed no impressive phenotype in the kidney.7 Therefore, the Fnip2 knockout mouse model was established; however, the Fnip2 knockout mouse did not show any phenotype, and survived until 3 years-of-age without any health problems.7 The homozygous whole-body Fnip1/Fnip2 double knockout mouse was embryonically lethal, whereas both of the whole-body heterozygous Fnip1/homozygous Fnip2 knockout mouse model and the whole-body homozygous Fnip1/heterozygous Fnip2 knockout mouse model are not embryonically lethal, suggesting that the complete loss of both Fnip1 and Fnip2 might mimic FlnC deficiency, and one allele of either Fnip1 or Fnip2 is sufficient to maintain Fnip function.7 The whole-body heterozygous Fnip1/homozygous Fnip2 knockout mouse model developed renal tumors at approximately 1.5 years-of-age, which was similar to those developed in whole-body heterozygous FlnC knockout mouse model and human BHD syndrome, suggesting that interaction between FlnC and Fnip1/Fnip2 is a key element for tumor suppression.7

Kidney-specific Fnip1/Fnip2 double knockout mouse model

Fnip1/Fnip2 doubly inactivated kidneys showed enlarged polycystic kidneys, which were similar to FlnC-deficient kidneys.1 Kidney-specific Fnip1/Fnip2 knockout mouse died at approximately 3 weeks-of-age, and Fnip1/Fnip2 doubly inactivated kidneys showed an activated mTORC1 signaling pathway and upregulated mitochondrial oxidative metabolism. Inactivation of FlnC in Fnip1/Fnip2 doubly inactivated kidneys did not cause any additional effect, supporting that Fnip1/Fnip2 doubly inactivated kidneys are totally identical to FlnC inactivated kidneys.7 Absolute Fnip2 mRNA copy number was low relative to Fnip1 in organs that showed phenotypes under Fnip1 deficiency, but was comparable with Fnip1 mRNA copy number in mouse kidney, suggesting that Fnip1/Fnip2 mRNA copy number ratio might be a determinant for the phenotypes of Fnip1 or Fnip2 knockout mouse model.7

Lung-specific FlnC knockout mouse model

The lung epithelial cell-specific FlnC knockout mouse model using the SP-C-rtTA/tetO-Cre transgenic mouse showed the apoptosis of lung epithelial cells, alveolar enlargement, and an impairment of both epithelial barrier and overall lung function.57 FlnC-null lung epithelial cells showed decreased AMPK activity and a forced activation of AMPK using 5-aminoimidazole-4-carboxamide ribonucleotide reversed the enlarged alveolar size and the impaired lung function.57 Whole-body heterozygous FlnC knockout mouse showed a trend toward increased elastance after mechanical ventilation.58 These mouse models are useful tools to clarify the mechanism of human BHD-associated lung cysts development.
Skin-specific \textit{Flnn} knockout mouse model

Skin-specific Flcn knockout mouse model using keratin 14 (\textit{K14})-Cre transgenic mouse showed delays in eyelid opening, wavy fur, hair loss and epidermal hyperplasia with increased levels of mTORC1 activity.\textsuperscript{59} plakophilin-4 (p0071), which regulates RhoA signaling, was identified as a Flcn binding partner, and loss of Flcn/p0071 function drives increased cell–cell adhesion, altered cell polarity and dysregulation of RhoA signaling.\textsuperscript{59,60}

Other in vivo models

The Nihon rat model, which develops preneoplastic lesions at 3 weeks-of-age and adenoma at 8 weeks-of-age, harbors a single nucleotide insertion within exon 3 of \textit{FLCN} (c.462_463insC), producing a frameshift and premature stop codon within the gene. Renal tumors that develop in the Nihon rat are largely composed of clear cell renal cell carcinoma and loss of heterozygosity of the \textit{FLCN} gene was observed in these tumors.\textsuperscript{61–63} A German shepherd harboring a H255R mutation in the \textit{FLCN} gene develops renal cystadenocarcinoma and nodular dermatofibrosis. Renal tumors are bilateral multifocal and loss of heterozygosity at the \textit{FLCN} locus was observed.\textsuperscript{64–67} Drosophila testis in which \textit{DBHD}, a drosophila homologue of \textit{FLCN}, was artificially deleted, showed overproliferations of germ cells, suggesting that \textit{DBHD} might be critical for stem cell maintainance.\textsuperscript{68}

Conclusions

BHD syndrome is an autosomal dominant hamartoma syndrome characterized by the development of renal tumors, fibrofolliculomas and lung cysts. The management of kidney cancer is important, and postoperative renal function as well as oncological radicality by surgery is critical for overall survival of BHD patients. A causative gene, \textit{FLCN} and its interacting partners, \textit{FNIP1} and \textit{FNIP2}, cooperatively play important roles in metabolic pathways including AMPK-mediated energy sensing, mTORC1-dependent cell proliferations and \textit{Pparge1a}-driven mitochondrial oxidation; the dysregulation of these metabolic pathways triggers aberrant kidney cell proliferations and renal tumorigenesis (Fig. 4). Deciphering how metabolic conversions under the functional loss of \textit{FLCN}/\textit{FNIP1}/\textit{FNIP2} complex trigger renal tumorige-

![Fig. 4 Schemes of the FLCN/FNIP1/FNIP2 pathway. Yellow and light blue show oncogene and tumor suppressors for kidney cancer, respectively.](image-url)
nosis might lead to the development of targeted therapeutics for kidney cancer patients.

Links for BHD research

The sites, BHD foundation (http://www.bhdsyndrome.org/) and BHD net (http://www.bhd-net.jp/) (currently Japanese only) provide useful information for BHD research.

Conflict of interest

None declared.

References

9 Baba M, Hong SB, Sharma N et al. Folluculin encoded by the BHD gene interacts with a binding protein, FNIP1, and AMPK, and is involved in AMPK and mTOR signaling. Proc Natl Acad Sci USA 2006; 103: 15552–7.
Birt-Hogg-Dube syndrome