The Pathologic and Molecular Genetic Landscape of the Hereditary Renal Cancer Predisposition Syndromes

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Abstract

It is estimated that 5-8% of renal tumors are hereditary in nature with many inherited as autosomal dominant. These tumors carry a unique spectrum of pathologic and molecular alterations, the knowledge of which is expanding in the recent years. Indebted to this knowledge, many advances in treatment of these tumors have been achieved. In this review, we summarize the current understanding of the genetic renal neoplasia syndromes, the clinical and pathologic presentations, their molecular pathogenesis, the advances in therapeutic implications and targeted therapy.
**Introduction**

The kidney and renal pelvis are the sixth leading site of new cancer diagnoses for men and the ninth for women according to American Cancer Society statistics.\(^1\) Most of these tumors arise from the renal tubules and occur in a sporadic basis, however, it is estimated that up to 5-8% are heritable.\(^2\) The knowledge about these heritable tumors is expanding, indebted to the advances in the genetic understanding of these tumors.\(^4\) To date, several genetic syndromes have been described with a high predisposition to the development of renal tumors (Table 1). In this review, we summarize the current understanding of the genetic renal neoplasia syndromes, the clinical and pathologic presentations, their molecular pathogenesis, and the advances in therapeutic implications and targeted therapy.

**Von Hippel–Lindau Disease**

Von Hippel–Lindau (VHL) disease is an autosomal dominant disease that was first described by Eugen von Hippel in 1904 in a patient with retinal angioma.\(^5\) In 1926, Arvid Lindau described angiomas of the cerebellum and spine, and later the term “Von Hippel–Lindau disease” was used in 1936.\(^6\) It wasn’t until 1993 when a series of genetic studies identified the VHL tumor suppressor gene to be responsible for the syndrome.\(^7-9\) It is mapped to the short arm of chromosome 3 (3p25.3) and encodes two pVHL proteins, a full length 213-amino acid protein (pVHL\(_{30}\)) and a small protein pVHL\(_{19}\). Both of these protein isoforms have similar effects.\(^10\) Patients with VHL disease have a germline inactivating mutation involving one of the VHL tumor suppressor genes.\(^11\) Contrasting with sporadic cases, familial cases require only “one-hit” for the disease development.\(^12\)

pVHL proteins are members of the ubiquitin ligase family. They play a key role in regulating hypoxia-sensing pathways, particularly in regulation of hypoxia-inducible factor (HIF).\(^13\) HIF is a transcription factor formed of a heterodimeric complex composed of α- and β-subunits which regulates the cellular hypoxic response through regulation of genes involved in energy metabolism, angiogenesis and apoptosis.\(^14-16\) Under normoxic cellular conditions, two HIF-α subunits are hydroxylated by a propyl hydroxylase (PHD) enzyme, which facilitates the binding of pVHL ubiquitin–ligase complex (pVHL, Elongin B, Elongin C, Rbx1, NEDD8, and Cullin-2) to the HIF-α subunits and targets them for proteasomal degradation through the process of polyubiquitylation. In hypoxic conditions, no hydroxylation of HIF-α subunits occurs, and therefore they are not directed
toward proteasomal degradation. Instead, they form stable complexes with HIF-β-subunits which in turn regulate gene expression though hypoxia-response element sequences. This results in activation of downstream genes, including glucose transporters (GLUT1), glycolytic enzymes, growth factors [vascular endothelial growth factors (VEGF), platelet derived growth factors (PDGF), transforming growth factor-α (TGF-α)], and ion transporters, which in turn execute the response to hypoxia (Figure 1). Similarly, in the absence of functioning pVHL, the HIFα is not degraded, regardless of the cellular oxygen content leading to HIF1α accumulation and upregulation of downstream genes, allowing for inappropriate overproduction of the hypoxia-inducible mRNAs. This in turn promotes angiogenesis, proliferation, and metabolism. The HIF α pathway also interacts with multiple intracellular pathways, including PI3K/AKT/MTOR, RAS-RAF-MEK-ERK, NF-kB, MITF and p53, epithelial–mesenchymal transformation, and chemokines such as CXCR4 and CCR7 (Figures 1 and 2).

There are three members in the HIF α family (HIF1α, HIF2α, and HIF3α). When bound to DNA, both HIF1α and HIF2α have transcriptional activation properties. They target some common genes, but the targets differ depending on the cellular context. In vitro studies identified HIF2α as the primary driver of oncogenesis in clear cell renal cell carcinoma (CCRCC); working as an oncogene, its accumulation was demonstrated in VLH-/- cell lines, and its overriding effect on pVHL function was identified in xenograft assay. On other hand, the lack of HIF1α was identified in clear cell carcinoma cell lines. Though the loss of this protein has not been identified in CCRCC, some observers speculate that haploinsufficiency may be sufficient to promote oncogenesis. As such, findings support that HIF1α works as a tumor suppressor gene. Genotypic-phenotypic correlation studies have led to the classification of VHL into types 1 and 2, where the former is associated with a low risk of developing pheochromocytoma. Type 2 is further divided into 3 subtypes: 2A (low risk of renal cell carcinoma), 2B (high risk of renal cell carcinoma) and 2C (pheochromocytoma risk only).

The manifestations of VHL disease include renal and non-renal manifestations. The renal manifestations include multiple bilateral renal cysts and clear cell renal cell carcinoma (CCRCC) which occur in 30-70% of patients, with the latter occurring in up to 70% of patients by the age of 60 years and is considered the leading cause of mortality in these patients. In these patients, tumors are
often multicentric and bilateral, and may develop intracystic or de novo. Microscopic analysis of the kidneys in these patients identified widespread clear cell abnormalities which are thought to be precursors for CCRCC. These clear cell abnormalities are extremely rare in sporadic cases. Classically, CCRCC exhibits variable architectural patterns, including nested, alveolar, and acinar patterns. These patterns are especially prominent in small multiple tumors associated with VHL syndrome (Figure 3). The tumor cells have clear cytoplasm and a distinct membrane; however, tumors with eosinophilic cytoplasm are not uncommon especially in high-grade tumors. Additionally, patients with VHL disease may develop tumors with small papillary tufts, branched tubules, branched papillae and apically aligned nuclei, features that resemble those seen in clear cell papillary renal cell carcinoma (CCPRCC). However, these tumors lacked the characteristic immunoprofile of sporadic CCPRCC with similar rate of chromosome 3p abnormalities to that seen in CCRCC.

Understanding the molecular pathways provided the foundation for developing new treatments for CCRCC. Monoclonal antibodies that block VEGF and tyrosine kinase inhibitors (TKIs) are approved treatments for advanced CCRCC. Other agents in development include drugs that target HIF2α. The small molecules PT2385 and PT2399 act as HIF2α antagonists that have shown effectiveness in blocking cancer growth; these molecules work by disrupting the formation of HIF2α and HIF-β dimerization and subsequently inhibiting the activation of downstream genes. In addition, recent studies have identified CCRCC as an immunogenic tumor with a high number of tumor-infiltrating lymphocytes, and recent trials combining anti-angiogenics and check-point inhibitors have shown promising results in the clinical trials.

The nonrenal manifestations include retinal and central nervous system hemangioblastomas (49-80%), pancreatic cysts or neuroendocrine tumors (35-70%), epididymal cystadenomas and broad ligament cystadenomas (25-60%), pheochromocytoma (10-20%), and endolymphatic sac tumors (6-5%) (Figure 2).

**Hereditary Papillary Renal Carcinoma**

Hereditary papillary renal carcinoma is an autosomal dominant syndrome. It is characterized by the development of multifocal and bilateral type 1 papillary renal cell carcinomas and has a high penetrance rate with an approximately 90% likelihood of developing RCC by age of 80 years.
Hereditary lesions are multiple and occur at earlier age compared with sporadic cases.\textsuperscript{32} Mutations of the MET proto-oncogene, receptor tyrosine kinase (\textit{MET}) gene have been associated with familial cases of this tumor and up to 13\% of sporadic cases.\textsuperscript{33, 34} The \textit{MET} gene is located on chromosome 7 (q21–q31) and spans more than 120 kb in length, consisting of 21 exons separated by 20 introns.\textsuperscript{35}

The \textit{MET} gene encodes a receptor tyrosine kinase that is activated by the hepatocyte growth factor (HGF) ligand which is secreted by mesenchymal cells, especially fibroblasts and smooth muscle cells. When secreted, it binds to the MET receptor and induces its dimerization, autophosphorylation and activation of multiple intracellular signaling pathways, including PI3K/AKT, JAK/STAT, Ras/MAPK, SRC, and Wnt/\beta-catenin (Figure 2).\textsuperscript{36} Activating the mutation of \textit{MET} gene, therefore, results in cellular proliferation, neovascularization, and cell motility.\textsuperscript{37} The mutant \textit{MET} allele is duplicated and overexpressed in tumor cells, which indicates that duplication of the mutant allele is necessary before the cell enters the tumorigenic pathway.\textsuperscript{38}

Histologically, hereditary papillary renal cell carcinomas share a similar morphology with sporadic tumors. However, hereditary cases commonly show multiple microscopic papillary lesions and adenomas involving the grossly unremarkable tissue. The tumors show papillary renal cell carcinoma type 1 with papillary formations lined by a single layer of small basophilic cells. The tumors usually have fibrous pseudocapsule especially when larger than 0.7 cm.\textsuperscript{32}

\textbf{Hereditary Leiomyomatosis and Renal Cell Carcinoma}

Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is another genetic syndrome with autosomal dominant inheritance. It was first reported in 2001 by Launonen et al.\textsuperscript{39} The genetic alteration in HLRCC involves the fumarate hydratase (\textit{FH}) gene at chromosome 1q42.3–q43. It is a tumor suppressor gene, that require “two-hit/ biallelic” inactivating mutation for tumor development, one-hit” being germline.\textsuperscript{40} It encodes fumarate hydratase enzyme, a critical enzyme involved in converting fumarate to malate in the tricarboxylic acid (TCA) cycle in the mitochondria. In addition to the mitochondria, this enzyme has been recently detected in the cytosolic and nuclear compartments of the cell. Therefore, the presence of mutated \textit{FH} gene leads to imbalance of fumarate levels in different cellular compartments affecting cellular metabolism, post-transcriptional protein modifications, gene expression, and DNA repair response. Within the mitochondria, lack of FH
enzyme leads to accumulation of fumarate, which competitively inhibits PHD enzyme, PHD is necessary for HIF hydroxylation creating a pseudohypoxic state, similar to that seen in VHL mutation, which promotes tumorigenesis through dysregulation of growth factors, glucose transporter, and glycolytic enzymes (Figure 2).\textsuperscript{41,42} In addition to the inhibition of the PHD enzyme, the lack of FH enzyme leads to metabolic transformation, shifts from oxidative phosphorylation to glycolysis and decreased AMP-activated kinase (AMPK) levels with activation of anabolic factors.\textsuperscript{43,44} Decreased intracellular AMPK levels leads to the reduction in divalent metal transporter (DMT1) “iron transporter” expression resulting intracellular iron deficiency. This in turn results in the activation of iron regulatory proteins, PHD enzyme inhibition, and increased expression and stabilization of the HIF-1α.\textsuperscript{45}

In addition, fumarate inhibits dioxygenases, including histone and DNA demethylases that maintain and regulate epigenetics and genomic integrity. In the nucleus, ten-eleven translocation (TET) enzymes, members of the dioxygenases enzymes family, catalyze the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine oxidized (5-hmC) leading to the demethylation of DNA. Acting as a TETs inhibitor, fumarate reduces the levels of 5-hmC contributing to DNA methylation. Recent methylation profile analysis has identified the majority of FH-RCC exhibiting global hypermethylation in CpG sites enriched in genes involved in DNA binding-transcription activity and tumor suppressor genes (CDKN2A, APC, MGMT, and TP53).\textsuperscript{46,47} Furthermore, fumarate can regulate methylation genes in immune cells controlling the expression of proinflammatory factors and immune pathways. Recent studies demonstrated that some renal FH-deficient renal cell carcinoma (FH-deficient RCC) exhibits immunogenic properties with increased levels of tumor-infiltrating lymphocytes and high expression levels of PD-L1, all in all.\textsuperscript{46,48,49} However, this finding has been not replicated by other studies which found weak to mostly negative staining by immunohistochemistry, although limited cases were analyzed thus far.\textsuperscript{50,51} In addition, altered histone methylation affects DNA repair by homologous recombination.\textsuperscript{52} Further studies showed that FH-deficient RCC carries a low somatic mutation burden but frequent copy number alteration, mainly involving loss of 1p, 8q, 9p, 10p, and 15q and gain of 4p, 7q, 11q, and 19p.\textsuperscript{46,53}

Patients with this syndrome are at high risk of developing renal and nonrenal manifestations, including multiple cutaneous and uterine leiomyomata, and an aggressive form of renal cell
carcinoma “HLRCC-associated RCC”. Several criteria were proposed for the clinical diagnosis, including a major criterion of multiple cutaneous leiomyomata, with at least one confirmed histologically; and multiple minor criteria including solitary cutaneous leiomyoma and family history of HLRCC, presence of severely symptomatic uterine leiomyoma/ta before the age of 40, papillary renal cell cancer before the age of 40 or first-degree family member who meets one of the above-mentioned criteria. Meeting the major criterion makes the diagnosis of HLRCC likely, while meeting two minor criteria should raise the suspicion of HLRCC.

The HLRCC-associated renal cell carcinoma is often unilateral, unifocal, and has a high propensity for metastasis. Morphologically, these tumors most commonly have similar features to papillary renal cell carcinoma, with the unique presence of prominent, inclusion-like eosinophilic nucleoli, surrounded by clear halos (Figure 4). Interestingly, the uterine leiomyomata also develop at a younger age and have similar prominent, inclusion-like eosinophilic nucleoli. Despite their helpfulness, their presence sometimes is only scattered in the tumor. Another helpful feature that should prompt work up is that these tumors are most often composed of different architectural patterns, including tubulopapillary, solid, or cystic pattern with frequent intracystic papillary growth. Additionally, tubulocystic carcinoma-like morphology with high grade features has been described in these tumors and should prompt additional work up.

Given this heterogeneous morphology, Skala et al., has proposed a practical diagnostic approach. Upon encountering a kidney tumor with wide morphologic spectrum with prominent nucleoli with perinucleolar halos (particularly when diffuse), the diagnosis of HLRCC should be suspected, especially in the presence of personal or family history of RCC or multiple leiomyomata. This is especially important when RCC developed at a younger age (<40 years). Judicious use of immunohistochemistry in these cases is warrant, and should demonstrates loss of FH and verexpression of modified cysteine-S-(2-succino)cystine. Additionally, for patients without previous diagnosis, communication with clinicians lays the best road map for managing these patients. Loss of H protein expression by immunohistochemistry is a good trigger for germline mutational testing. However, until the diagnosis is confirmed by the gold standard “germline mutation” test, the diagnosis of FH-deficient RCC should be used.
Based on the molecular mechanisms associated with FH mutations and the results of the phase II clinical trial (NCT01130519), the National Comprehensive Cancer Network has endorsed the combination of bevacizumab and erlotinib (anti-VEGFR and anti-EGFR; respectively) for treatment of HLRCC; however, the above molecular mechanisms open the doors for more frontline therapeutic options such as checkpoint inhibitors, DNA hypomethylating agents, some of which are under consideration/investigation for this aggressive tumor.\(^{51}\)

Tuberous Sclerosis

Tuberous sclerosis complex (TSC) is a multisystem genetic disorder characterized the growth of hamartomas and multiple benign tumors in different parts of the body. It is estimated to affect 1 in 6,000 people and is inherited as an autosomal dominant disease with near-complete penetrance and variable expressivity; however, up to two-thirds are de novo mutations.\(^{58,59}\)

TSC is associated with mutations of TSC complex subunits 1 or 2 genes (TSC1 or TSC2) located at chromosomes 9q34 and 16p13.3, and encodes hamartin and tuberin proteins, respectively. They both are tumor suppressor genes and inactivation of both alleles “two-hit” are required for tumor development. Similar to VHL disease and HLRCC, one “hit” is being germline and the second is “somatic” inactivation. A wide spectrum of mutations has been reported in patients with TSC.\(^{60}\)

Normally, TSC1 (hamartin) and TSC2 (tuberin) form a heterodimer complex.\(^{61}\) The signaling pathways through this complex have multiple phosphorylation sites; those on TSC1 site are GSK3β (glycogen synthase kinase 3β) and CDK1 (cyclin dependent kinase 1), while ERK1/2 (extracellular signal-regulated kinase 1 and 2), RSK1 (p90 ribosomal protein S6 kinase 1), MK2, Akt (protein kinase B), AMPK (adenosine monophosphate-activated protein kinase) and GSK3β are on TSC2 site.\(^{62}\)

Most of the TSC functions are related to the GAP domain of TSC2. Upon stimulation, the TSC1/TSC2 complex exerts its effect through the RHEB (Ras homolog, MTORC1 binding)-GTP. After growth factor stimulation, the TSC1/TSC2 complex is phosphorylated, leading to a decrease in its GTPase-activating activity.\(^{62}\) The activated RHEB (RHEB -GTP bound) stimulates MTOR (mechanistic target of rapamycin kinase) which leads to phosphorylation cascades resulting in cell
growth and proliferation. Similarly, lack of TSC1/TSC2 complex function due to genetic alterations leads to constant dysregulated activation of the RHEB and MTOR pathway (Figure 2).  

Patients with TSC develop several renal and extrarenal manifestations. The renal manifestations occur in up 90% of patients by adulthood, and include renal cystic disease, angiomyolipoma, oncocytoma and renal cell carcinoma (Table 2). The renal cystic disease affects up to 45% of patients and ranges from microcystic (undetectable by routine imaging) to larger cysts. Cases of glomerulocystic kidney disease and polycystic kidney disease have also been associated with SC syndrome. Interestingly, this phenotype is mostly caused by larger deletion events involving the TSC2 and PKD1 genes on chromosome 16p13, causing the so-called “TSC2/PKD1 contiguous gene deletion syndrome”. Renal angiomyolipoma (AML) is another major renal manifestation of TSC syndrome, occurring in up to 80% of patients. It often appears as multiple bilateral tumors with a rapid growth during childhood and adolescence which then stabilizes during adulthood. The abnormally developed vasculature in angiomyolipoma is associated with aneurysmal development, putting TSC syndrome patients at risk of hemorrhage. Both classic and epithelial AMLs are identified in TSC patients, with the former composed of the three components (thick-walled blood vessels, adipose tissue and spindle-polygonal (epithelial) smooth muscle) in variable proportions, while the epithelial morphology predominates (>80% of the tumor) in the epithelial type. MTOR inhibitors have been approved as the first-line treatment of TSC-associated AMLs that measure > 3 cm and AMLs in patients who are not candidates for surgical treatment. In recent years, everolimus and other MTOR inhibitors have been recommended as first-line treatment of TSC-related AML as they have been shown to achieve satisfactory results.

The risk of RCC development appears similar to that of the general population, however, these occur at a significantly younger age. Unlike many hereditary renal cancer syndromes, patients with SC syndrome develop several histologic subtypes of renal epithelial tumors, including tumors with clear-cell or eosinophilic/oncocytic morphology. The most distinctive entities are low-grade oncocytic tumors, renal cell carcinoma with fibromyomatous stroma (RCC-FMS), and eosinophilic solid and cystic RCC (ESC-RCC) (Figures 5 and 6). Low-grade oncocytic tumor is a recently proposed renal tumor that has been described in non-syndromic settings, however, cases with multiple tumors were described in the setting of TSC. They usually have solid architecture with eosinophilic cells,
round to oval 'low-grade' nuclei, without nuclear irregularities and showing focal perinuclear halos.\textsuperscript{77} RCC-FMS was found to be the most prevalent type of and is characterized by nodules of solid nests and papillae surrounded by prominent fibromuscular stroma with the tumors cells exhibiting clear to pale eosinophilic cytoplasm. The ESC-RCC shows solid and cystic growth with the lesional cells being eosinophilic and characterized by distinctive cytoplasmic granules. Vacuolated eosinophilic tumor (EVT), an emerging entity harboring \textit{TSC2} and \textit{MTOR} inactivation mutations in the sporadic cases, has been also documented in few patients with TSC syndrome. This tumor is characterized by solid nests of eosinophilic cells with cytoplasmic vacuolation and prominent nucleoli. Additionally, patients with TSC syndrome can develop tumors with overlapping features between oncocytoma and chromophobe renal cell carcinoma (ChrRCC).\textsuperscript{78, 79}

On the other hand, the nonrenal manifestations seen in patients with TSC syndrome include skin lesions (facial angiofibromas, periungual fibromas, shagreen patches, and hypopigmented macules), central nervous system lesions (cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas), cardiac rhabdomyomas, pulmonary lymphangioleiomyomatosis, and retinal astrocytic hamartomas.

\textbf{Birt–Hogg–Dubé Syndrome}

Birt–Hogg–Dubé (BHD) syndrome is a rare genetic disease that is estimated to affect 1 in 200,000 people. It is inherited as an autosomal dominant disease with incomplete penetrance. These patients are at higher risk for the development of several renal and nonrenal manifestations.

BHD syndrome is associated with mutations of folliculin gene (\textit{FLCN}) or \textit{BHD} at 17p11.2, which codes for a folliculin protein with a tumor suppressor function.\textsuperscript{80} Alterations of the \textit{FLCN} can occur due to point mutations, insertions, deletions, and putative splice-site mutations.\textsuperscript{81} In the majority of cases, mutations are nonsense, resulting in truncation and dysfunctional gene products. Normally, folliculin interacting proteins 1 and 2 (FNIP1 and 2) bind to the C-terminus of the FLCN protein.\textsuperscript{82} This complex then interacts with \textit{5' AMP-activated protein kinase} (AMPK) related to the energy- and nutrient-sensing pathways.\textsuperscript{83} In a low energy state, AMPK is phosphorylated by LKB1 and calmodulin kinase, which in turns lead to negative regulation of \textit{MTOR} either directly or through TSC2 phosphorylation, leading to inhibition of protein synthesis and cell growth (Figure 2).
Additionally, activated AMPK upregulate and directly phosphorylate PGC-1α/PPARGC1A (PPARG oactivator 1 α), a transcription factor regulating mitochondrial biogenesis. Interestingly, alterations in this mitochondrial gene, in addition to TFAM (transcription factor A, mitochondrial) transcription factor gene, are only seen in BHD-associated tumors. The loss of FLCN function promotes the MTORC1 and MTORC2 overactivation and upregulation of PGC-1α/PPARGC1A gene expression leading to cellular proliferation and tumor development. Furthermore, FNCL is involved in the amino acid-induced activation of the MTORC1 pathway, which has been linked to the pathogenesis of this syndrome. MTORC1 exerts its function through three main downstream targets, TFEB, 40S ribosomal S6 Kinases (S6K), and the transition initiation factor-binding protein 1 (4E-BP-1). In the amino acid-induced activation pathway, sensing the amino acid levels within the lysosome leads to activation of Rag GTPase heterodimers RagsA/B and RagsC/D by GTPase activator protein (GATOR1) and FLCN, respectively. Active Rags attract MTORC1 to the lysosomal membrane. While RagsA/B facilitates MTORC1-RHEB interaction, RagsC/D facilitates FCLN-dependent MTORC1-mediated phosphorylation of the transcription factor TFEB which results in TFEB entrapment in the cytoplasmic subcellular compartment. The latter interaction is not required for the phosphorylation of the other MTORC1 substrates. Loss of FNCL causes the loss of TFEB inhibition. Constitutively active TFEB moves to the nucleus resulting in activation of MTORC1 via increased expression of the RagC/D heterodimer. When MTOC1 is activated, it phosphorylates S6K2 and 4EBP-1 which are involved in cellular proliferation. Studies of mice models have identified that the constitutive activation of TFEB is the main driver of kidney abnormalities in BHD. MTOR inhibition with everolimus treatment may be clinically effective in the treatment of BDH-associated carcinoma.

The renal manifestations include several tumors of different histologic subtypes and develop round the age of 40. These may occur unilaterally or bilaterally, unincertic or multicentric within the same kidney. “Hybrid” tumor with overlapping features between ChrRCC and oncocyoma is the most common tumor seen in these patients. The ChrRCC-like component is identical to those seen in nonsyndromic cases; however, the oncocyoma-like component is formed of compact nested architectures, lacking the loose connective tissue stroma and central scar. Additionally, hybrid tumors may present with EVT-like morphology and immunohistochemistry (discussed in the TSC
The term “hybrid oncocytic tumor” should be restricted to multiple and/or bilateral hereditary cases, while the term “oncocytic renal neoplasm of low malignant potential, not further classified” should be reserved for solitary and sporadic cases. Typically, ChrRCC is the second most common type; however, depending on the population examined, it was found to be the most common tumor in the Japanese population, although the difference was not prominent. Additionally, renal oncocytosis, defined as poorly circumscribed microscopic lesions that tend to expand into renal tubules and has morphologic appearance of renal oncocytoma. A clinical association between these lesions and multiple larger tumors was described in BHD syndrome patients. Clear cell renal cell carcinoma, papillary renal cell carcinoma and renal oncocytoma are other renal manifestations that may develop in these patients, although the frequency of these tumors is low and may mirror that seen in the general population.

The nonrenal manifestations including multiple benign cutaneous tumors (fibrofolliculomas in the forehead, scalp, face and neck; trichodiscomas, and acrochordons), pulmonary cysts, and medullary thyroid carcinoma.

Several diagnostic criteria were proposed for the diagnosis of BHD, the presence of one or more is suggestive of BHD syndrome. First, ≥2 cutaneous papules clinically consistent with fibrofolliculoma and/or trichodiscoma and ≥1 biopsy confirmed fibrofolliculoma. Second, multiple bilateral pulmonary cysts located mainly in the basilar regions of the lung with or without a history of spontaneous pneumothorax that develops before 40 years of age, but especially with a family history of these pulmonary manifestations. Third, bilateral, multifocal ChrRCC or hybrid oncocytic tumors especially with the presence of a family history of renal tumors or onset before the age of 50 years. Lastly, a combination of these cutaneous, pulmonary or renal manifestations in the patient or one of their family members. A definitive diagnosis is made by a genetic test demonstrating a germline FLCN mutation. Therefore, a diagnostic approach similar to that proposed for HLRCC should be implemented, and should prompt a direct communication with the clinician about the possibility of BHD syndrome.

**Succinate Dehydrogenase Germline Mutations**

Succinate dehydrogenase (SDH) is an enzyme complex that is bound to the inner
mitochondrial membrane and participates in the TCA cycle and electron transport chain, necessary for energy production. The SDH enzyme consists of 4 subunits, which are encoded by 4 mitochondrial complex II genes (SDHA, SDHB, SDHC and SDHD), located on chromosomes 5p15, 1p36, 1q21, and 11q23, respectively, along with 2 assembly factors (SDHAF1 and SDHAF2) located on chromosomes 9q13 and 11q12, respectively.

Functionally, SDHA oxidizes succinate to fumarate in the TCA cycle. SDHB is involved in the mitochondrial oxidative phosphorylation complex for energy production while SDHC and SDHD are responsible for anchoring the SDH protein complex to mitochondria. Germline mutations in any of these genes are inherited as autosomal dominant syndromes that are associated with pheochromocytoma/paraganglioma (PGL). The pathogenic role of SDH mutation in tumorigenesis is not fully understood; however, the accumulation of intracellular succinate is thought to be related to an oncogenic effect caused by PHD enzyme inhibition and genome-wide hypermethylation (Figure 2). As such, there are no evidence-based treatment/guidelines for targeting unresectable or advanced tumors. It is worth noting that despite the autosomal dominant mode of inheritance, mutations in genes of each SDH complex component have varying penetrance patterns depending on genes expression and epigenetic modifications; for example, mutations involving SDHB and SDHC show a high rate of penetrance, whereas SDHD and SDAF2 mutations show maternal imprinting in most families with paternal-origin expression. On the other hand, mutations involving the SDHA gene show low penetrance and are rarely involved in hereditary cases; however, distinct variants are common in populations, and the rate of polymorphism is increasingly detected with recent NGS approach. These findings highlight the need to characterize further of the epigenetic changes and molecular pathways for each component to guide treatment, surveillance, and genetic counseling.

SDH-deficiency associated syndromes are characterized by the development of renal tumors and/or extrarenal pheochromocytoma/paraganglioma and gastrointestinal stroma tumors (SDHB-deficient GIST; GIST type 2) with variable frequency between affected genes. SDH-deficient renal cell carcinomas have distinct morphology. They are characterized by variable architectural patterns, including solid, tubular, and cystic patterns. The cells are uniform with eosinophilic cytoplasm and intracytoplasmic vacuoles or flocculent inclusions, representing abnormal mitochondria with excess matrix and few cristae. Entrapped non-neoplastic renal tubules is a common finding in these lesions.
In addition to these common features, SDH-deficient RCC has been reported to have high grade and sarcomatoid areas (WHO/ISUP nucleolar grade 3-4). The cytoplasm has denser eosinophilic quality with darker and coarser chromatin pattern. Solid sheet-like growth, irregular anastomosing tubular structures, and papillary architectures embedded in desmoplastic stroma were also observed in high grade tumors. Tumors develop most commonly in patients with germline mutations in SDHB, less frequently in SDHC and SDHD, and only rarely in SDHA. Therefore, loss of SDHB reaction by immunohistochemistry with retained reaction in the non-neoplastic entrapped tubules serves as a valuable tool to the diagnosis (Figure 7B). This loss of reaction can also be seen in non-renal manifestations in SDH deficient patients.

**Constitutional Chromosome 3 Translocations**

Constitutional chromosome 3 translocation has also been found to be associated with an increased risk of developing bilateral and multifocal clear cell RCC. In 1979, Cohen et al described 10 cases of renal cell carcinoma occurring in 3 consecutive generations of a family member. Karyotyping showed balanced reciprocal translocation between chromosomes 3 and 8, t(3;8)(p21;q24), occurring equally between both genders. Subsequently, several constitutional translocation associated RCC have been described. To date, 19 chromosomal rearrangements involving chromosome 3 have been described. Molecular analysis of these showed disruption of a series of tumor suppressor genes, none of which were in the 3p location. These rearrangements and the affected genes are summarized in Table 3.

**BAP1 mutations and familial kidney cancer**

*BAP1* (BRCA1 associated protein-1) is a tumor suppressor gene that is located on the short arm of chromosome 3 (3p21). It encodes a nuclear deubiquitinase and is involved in regulation of transcription, cell cycle and growth, and response to DNA damage (Figure 2).

Somatic *BAP1* mutations were identified in uveal melanoma, mesothelioma and clear cell RCC. In the latter, it was found to be associated with high WHO/ISUP grade and poor prognosis. On the other hand, germline mutations are inherited as an autosomal dominant syndromes that
predispose patients to develop multiple tumors, including uveal melanoma, cutaneous melanocytic tumors (melanoma and atypical Spitz nevi), mesothelioma and RCC. The phenotype of RCC in individuals with \textit{BAP1} mutations has not been fully elucidated due to the small number of reported cases; however, both clear cell and non-clear cell RCCs have been described.\textsuperscript{129, 130}

\textbf{Cowden syndrome (PTEN hamartoma tumor syndrome)}

Cowden syndrome (CS) is inherited as an autosomal dominant syndrome that is caused by germline alterations of the phosphatase and tensin homolog (\textit{PTEN}) tumor suppressor gene located at chromosome 10q23.31. It is found in up to 70\% of affected individuals.\textsuperscript{131} Through antagonizing the function of PI3K and blocking the activation of downstream signaling events including MTOR, it is involved in the inhibition of cell cycle progression, induction of cell death, and modulation of arrest signals (Figure 2).\textsuperscript{132, 133}

CS is characterized by the development of multiple hamartomas and associated with an increased risk of different malignancies, including breast, endometrium and thyroid.\textsuperscript{134} The risk of RCC development in patients with CS is probably underestimated. Mester et al has described a >30-fold increased risk of RCC development in a cohort of CS patients and similar to \textit{BAP1} mutations, no specific phenotypic subtype has been described in individuals with \textit{PTEN} mutation.\textsuperscript{135, 136}

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Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References:


61. Plank TL, Yeung RS, Henske EP. Hamartin, the product of the tuberous sclerosis 1 (TSC1) gene, interacts with tuberin and appears to be localized to cytoplasmic vesicles. Cancer Res. 1998;58:4766-4770.


122. Yusenko MV, Nagy A, Kovacs G. Molecular analysis of germline t(3;6) and t(3;12) associated with conventional renal cell carcinomas indicates their rate-limiting role and supports the three-hit model of carcinogenesis. Cancer Genet Cytogenet. 2010;201:15-23.


Figure Legends

Fig 1. A summary of signal transduction pathways associated with pVHL.

Fig 2. A summary of signal transduction pathways associated with hereditary renal cell carcinoma syndromes.

Fig 3. A small unencapsulated focus of clear cell renal cell carcinoma in a patient with VHL disease.

Fig 4. Histologic images of hereditary leiomyomatosis and renal cell carcinoma associated-RCC showing large (inclusion-like) nucleoli (A) with associated loss of fumarate hydratase staining by immunohistochemistry (B).

Fig 5. Histologic images of eosinophilic solid and cystic renal cell carcinoma with formed of cells with clear to eosinophilic cytoplasm, and containing basophilic granules (A, B).

Fig 6. A histologic image of renal cell carcinoma with fibromyomatous stroma showing tumor nodules separated by fibromuscular stroma.

Fig 7. Histologic images of succinate dehydrogenase deficient renal cell carcinoma showing sheets of cells with eosinophilic, vacuolated cytoplasm and benign tubules at the periphery (A), and corresponding loss of succinate dehydrogenase staining by immunohistochemistry (B).

Table 1: A summary of the clinical and genetic findings in hereditary cancer syndromes of the kidney

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Mode of inheritance</th>
<th>Chromosomal location</th>
<th>Gene</th>
<th>Renal manifestations</th>
<th>Extrarenal manifestations</th>
</tr>
</thead>
</table>

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<table>
<thead>
<tr>
<th>#</th>
<th>Syndrome</th>
<th>Type</th>
<th>Chromosome</th>
<th>Gene(s)</th>
<th>Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Von Hippel–Lindau Disease</td>
<td>Autosomal dominant</td>
<td>3p25.3</td>
<td>VHL</td>
<td>Renal cysts (Multiple and bilateral) - CCRCC (Multiple and bilateral) - Hemangioblastoma (Retinal and CNS) - Endolymphatic sac tumors - Pancreatic cysts - Pancreatic NET - Epididymal cystadenomas in males - Broad ligament cystadenomas in females - Pheochromocytoma</td>
</tr>
<tr>
<td>2</td>
<td>Hereditary Papillary Renal Carcinoma</td>
<td>Autosomal dominant</td>
<td>7q21–q31</td>
<td>MET</td>
<td>PRCC-1 (Multiple and bilateral) None</td>
</tr>
<tr>
<td>3</td>
<td>Hereditary Leiomyomatosis and Renal Cell Carcinoma</td>
<td>Autosomal dominant</td>
<td>1q42.3–q43</td>
<td>FH</td>
<td>HLRCC-associated renal cell carcinoma Cutaneous and uterine leiomyomata</td>
</tr>
<tr>
<td>4</td>
<td>Tuberous Sclerosis</td>
<td>Autosomal dominant</td>
<td>9q34 and 16p13.3</td>
<td>TSC1 and TSC2</td>
<td>Renal cystic disease - Angiomyolipoma - Oncocytoma - CCRCC - Chromophobe-like RCC - Renal cell carcinoma with fibromyomatous stroma - ESC-RCC - EVT - Cutaneous lesions (facial angiofibromas, periungual fibromas, shagreen patches, and hypopigmented macules) - CNS lesions (cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas) - Cardiac rhabdomyomas - Pulmonary lymphangiopleiomyomatosis - Retinal astrocytic hamartomas</td>
</tr>
<tr>
<td>5</td>
<td>Birt–Hogg–Dubé Syndrome</td>
<td>Autosomal dominant</td>
<td>17p11.2</td>
<td>FLCN</td>
<td>“Hybrid” tumors - ChrRCC - Renal oncocytosis - Cutaneous tumors (fibrofolliculomas; trichodiscomas, acrochordons) - Pulmonary cysts - Medullary thyroid carcinoma</td>
</tr>
<tr>
<td>6</td>
<td>Succinate Dehydrogenase (SDH) Germline Mutations</td>
<td>Autosomal dominant</td>
<td>5p15, 1p36, 1q21, 11q23</td>
<td>SDHA, SDHB, SDHC, SDHD</td>
<td>SDH-deficient RCC - Pheochromocytoma/paraganglioma - SDHB-deficient GIST</td>
</tr>
<tr>
<td>7</td>
<td>Constitutional Chromosome 3 Translocations</td>
<td>Autosomal dominant</td>
<td>See table 2</td>
<td>See table 2</td>
<td>CCRCC (Multiple and bilateral) None</td>
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<td>8</td>
<td>BAP1 mutations and familial kidney</td>
<td>Autosomal</td>
<td>3p21</td>
<td>BAP1</td>
<td>no specific subtype - Uveal melanoma - Cutaneous melanocytic tumors</td>
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<tr>
<td>Renal cystic disease</td>
<td>Renal cystic disease</td>
<td>Characteristic Histologic Features</td>
<td>Characteristic Immunohistochemical Features</td>
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<td></td>
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<td>---------------------</td>
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<td>----------------------------------</td>
<td>---------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Renal cystic disease</td>
<td>Single or multiple cysts lined by plump eosinophilic cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TSC2/PKD1 contiguous gene syndrome: numerous cysts with flattened, cuboidal, and eosinophilic epithelial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glomerulocystic kidney disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiomyolipoma</td>
<td>Angiomyolipoma</td>
<td>Triphasic:</td>
<td>Cathepsin-K PAX8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thick-walled blood vessels</td>
<td>Melan-A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smooth muscles; epithelioid morphology is common</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adipose component</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Background: AML tumorlets and cysts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal cell carcinoma with fibromyomatous stroma</td>
<td>Nodules of solid nests, elongated tubules, and focal papillary structures</td>
<td>PAX8</td>
<td>Keratin 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cells with clear to pale eosinophilic cytoplasm</td>
<td>Keratin 7 (diffuse)</td>
<td>TFE3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abundant fibromuscular stroma</td>
<td>CD10</td>
<td>AMACR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Eosinophilic solid and cystic renal cell carcinoma | Macrocystic | CD10 (negative to focal) |
| | Microscopic: - Solid and macrocystic and microcystic growth - Eosinophilic cells - Abundant cytoplasm with distinct granules | PAX8 | Keratin 20 |
| | | Keratin 7 (can be only focally positive) |

| Eosinophilic vacuolated tumor | Solid, nested architecture | PAX8 | CD117 |
| | Vacuolated eosinophilic cytoplasm | Keratin 20 |
| | Prominent nucleoli | Cathepsin K |
| | | CD10 |

<p>| Tumors with oncytic morphology | Overlapping features between eosinophilic chromophobe renal cell carcinoma and oncocytoma | PAX8 | CD10 (negative to focal) |
| | | Keratin 20 |
| | | Melan-A |
| | | HMB45 |
| | | TFE3 |
| | | Keratin 7 (variable from negative to scattered positive cells only) |</p>
<table>
<thead>
<tr>
<th>Rearrangement</th>
<th>Affected gene</th>
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<tbody>
<tr>
<td>t(1;3)(q32;q13.3)</td>
<td>\textit{NORE1A} &amp; \textit{LSAMP}</td>
</tr>
<tr>
<td>t(2;3)(q33;q21)</td>
<td>\textit{DIRC1}</td>
</tr>
<tr>
<td>t(2;3)(q35;q21)</td>
<td>\textit{DIRC2} &amp; \textit{DIRC3}</td>
</tr>
<tr>
<td>t(2;3)(q37.3;q13.2)</td>
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<tr>
<td>t(2;17)(q21.1;q11.2)</td>
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<tr>
<td>t(3;4)(p13;p15)</td>
<td>\textit{KCNIP4}</td>
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<tr>
<td>t(3;4)(p13;p16)</td>
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<tr>
<td>t(3;4)(q21;q31)</td>
<td>\textit{FBXW7}</td>
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<tr>
<td>t(3;6)(p13;q25.1)</td>
<td>\textit{STXBP5}</td>
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<tr>
<td>t(3;6)(p14.2;p12)</td>
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</tr>
<tr>
<td>t(3;6)(q12;q15)</td>
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</tr>
<tr>
<td>t(3;6)(q11.2;q13)</td>
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</tr>
<tr>
<td>13</td>
<td>t(3;6)(q22;q16.2)</td>
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<td>14</td>
<td>t(3;8)(p14.2;q24.1)</td>
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<tr>
<td>5</td>
<td>t(3;12)(q13.2;q24.1)</td>
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<td>t(3;14)(q13.3;q23)</td>
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<tr>
<td>17</td>
<td>t(3;15)(p11;q21)</td>
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<td>8</td>
<td>ins(3;13)(p24.2;q32q21.2)</td>
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<tr>
<td>19</td>
<td>inv(3)(p14.2q12)</td>
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