Robert A. Figlin · W. Kimryn Rathmell
Brian I. Rini   *Editors*

Renal Cell Carcinoma

Translational Biology, Personalized Medicine, and Novel Therapeutic Targets

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Preface

Tremendous strides have been made in recent years in unraveling the aberrant biology driving renal carcinomas. These discoveries have led to unparalleled shifts in the treatment paradigm of this once devastating disease, historically known for its unrelenting progression of metastatic disease and high incidence of drug resistance and fatality. Rapidly emerging new therapeutic strategies that have the ability to neutralize this cancer have now engendered hope and optimism. In many instances, the speed of advances in clinical management has outpaced the biology, and observations made in the clinic using new therapeutics have fueled scientific discovery. In this way, renal cell carcinoma has truly served as a paradigm tumor type in the rapid flux of discovery from bench to bedside and back to the bench.

This textbook reviews and examines this enormously productive period with chapters touching on every major topic area in the modern era of renal carcinoma biology and treatment. Beginning with the discovery of the von Hippel Lindau (*VHL*) gene in 1993, we now understand on a more fundamental level the association of *VHL* mutation and the resultant HIF family stabilization as well as the intimate relationship this axis plays in the development of clear cell renal cell carcinoma. The unique and intricate genetics of this cancer are highly distinct from most other tumors, and the advances made in this cancer beyond *VHL* biology have been intrinsically driven by discoveries from familial renal cell carcinomas linked with newer large-scale genomic efforts in sporadic disease. The remarkable parallels of sporadic and familial diseases have enabled the elucidation of critical pathways in the renal tumorigenic process. These genetic findings fuel strategies to analyze and define sporadic tumors for greater accuracy in prognosis and prediction of response to therapy, the latest of which will be detailed in this text. Completing the circle, major new therapeutic strategies harness these biological discoveries, in particular angiogenic, energy metabolism, chromatin remodeling, tumor microenvironment, and classical signaling pathways. Many of these therapies have moved beyond management of metastatic disease to arenas in combination with surgical approach to advance the opportunities for durable remission or cure. This text will bring all of these avenues of investigation together for readers interested in understanding the

dynamics of this field in the last decade and anticipating a continued steep trajectory in advancements toward the cure of this disease in its many manifestations.

The field does continue to evolve at an enormously rapid pace. In addition to placing each of these major advances in historical context, the chapters in this textbook take a critical look forward to consider the future advances in each topic. The goal of this textbook is to educate the reader regarding the state of the art in renal cancer biology and therapeutic strategies as well as to engage readers as participants in an ongoing and exciting period of discovery and translation to advance the care of patients with renal cell carcinoma.

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Part I
Biology of Renal Cell Carcinoma

Chapter 1
The Genetic Basis of Kidney Cancer and Implications for Targeted Therapies

Laura S. Schmidt, Ramaprasad Srinivasan, and W. Marston Linehan

1.1 Introduction

Kidney cancer or renal cell carcinoma (RCC) comprises approximately 4% of adult malignancies and is the tenth leading cause of cancer-related deaths in the United States. It is estimated that over 58,000 new cases of kidney cancer were diagnosed in the USA in 2010 with greater than 13,000 cases resulting in death [1]. Although asymptomatic tumors are frequently detected during incidental imaging, the paucity of early warning signs contributes to the fact that nearly a third of cases are metastatic upon diagnosis with 5-year survival estimated at <10% for stage IV disease [2]. Although immunotherapy is effective in a small proportion of patients, the majority of advanced kidney cancer patients do not benefit from this approach. This underscores the need for reliable biomarkers for early detection and intervention and a better understanding of the pathways involved in kidney cancer to enable development of effective molecularly targeted therapies.

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Although kidney cancer was historically considered a single disease, we now know that it is a diverse group of kidney epithelial tumors, each characterized by a distinct histology, a unique clinical presentation and course, a different response to therapy, and associated with different genetic alterations. Using genetic linkage analysis approaches, the genes responsible for the six major inherited kidney cancer syndromes, von Hippel–Lindau (VHL), hereditary papillary renal carcinoma (HPRC), Birt–Hogg–Dubé (BHD) syndrome, hereditary leiomyomatosis and renal cell carcinoma (HLRCC), succinate dehydrogenase (SDH)-associated familial kidney cancer, and tuberous sclerosis complex, have been identified (Table 1.1). Subsequent studies from a number of research groups over the past two decades have contributed to our understanding of the processes involved in kidney tumor initiation and progression [3]. The finding that kidney cancer is a disease of cell metabolism and the elucidation of the metabolic pathways dysregulated by cancer gene mutations have fueled the development of promising targeted agents for kidney cancer treatment.

1.1.1 Inherited Clear Cell Kidney Cancer: von Hippel–Lindau

VHL is an autosomal dominantly inherited multisystem disorder in which patients develop cysts and clear cell tumors in the kidney (Fig. 1.1a–c), pancreatic neuroendocrine tumors and cysts, CNS hemangioblastomas, retinal angiomas, pheochromocytomas, endolymphatic sac tumors, and cystadenomas in the epididymis and broad ligament. VHL occurs in about 1/36,000 individuals with approximately 25–45% of VHL patients presenting with kidney tumors at a mean age of diagnosis of ~40 years. Individuals who inherit germline mutations in the *VHL* tumor suppressor gene on chromosome 3p25 are highly likely to develop phenotypic manifestations of VHL by age 70 [4, 5].

With the introduction of quantitative Southern blotting techniques, *VHL* mutation detection rate in VHL disease now approaches 100% [6]. The *VHL* mutation spectrum found in VHL patients includes point mutations resulting in protein truncation (frameshift, nonsense, splice site) and amino acid substitution (missense) (Fig. 1.1d) as well as whole and partial gene deletions, and interesting genotype–phenotype correlations are emerging. VHL disease has been subclassified based on the absence (type 1) or presence (type 2) of pheochromocytomas. Type 2 VHL is further subclassified based on low (type 2A) or high (type 2B) risk for kidney cancer; type 2C VHL is characterized by the presence of pheochromocytomas without other manifestations of VHL such as kidney cancer or hemangioblastomas [7, 8]. Mutations that are predicted to lead to complete loss of pVHL function (truncating mutations) or those which severely alter its structural integrity are associated with type 1 disease [7, 9], whereas missense mutations that would cause more subtle changes in pVHL function are more common in type 2 disease [10, 11]. Additionally, evidence suggests that VHL patients who inherit complete *VHL* gene deletions including the adjacent *HSPC300* gene have a lower risk for developing kidney cancer than those with partial gene deletions [12].
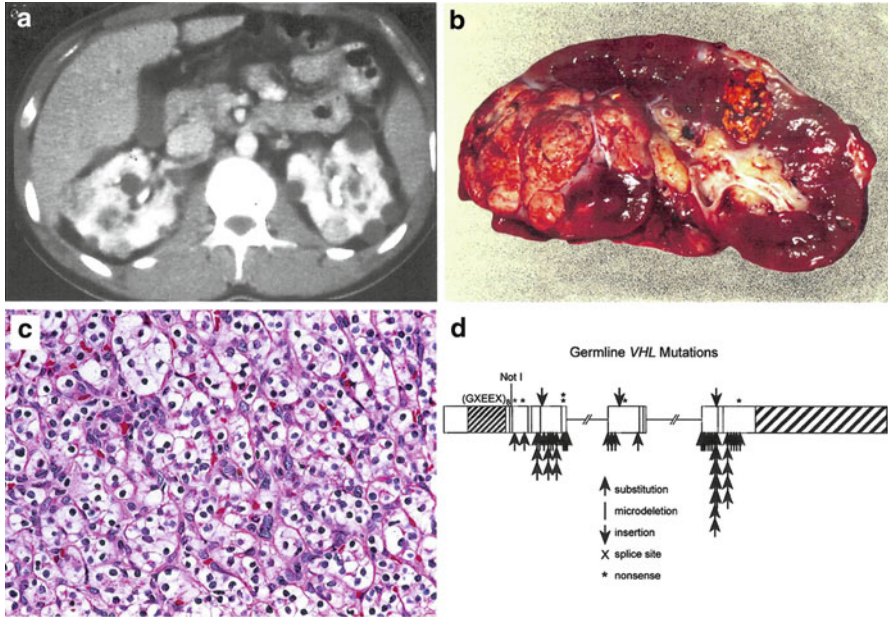
Fig. 1.1 Clear cell kidney cancer in VHL. Patients with VHL are at risk for bilateral, multifocal kidney cancer (**a** and **b**) with clear cell histology (**c**). ×40 magnification. *VHL* mutations in all three exons have been identified in the germline of affected individuals from VHL families (**d**). Used with permission [127]

1.1.2 Inherited Clear Cell Kidney Cancer: Translocation Families Involving Chromosome 3p

In 1979, Cohen and colleagues described a family with a balanced constitutional translocation involving chromosomes 3 and 8. At-risk family members who inherited the t(3;8)(p14;q24) translocation developed clear cell kidney tumors [13]. *VHL* mutations were identified in a subset of those tumors with concomitant loss of the derivative chromosome bearing the remaining *VHL* allele [14]. Additional constitutional chromosome 3 translocation families with a predisposition to develop kidney cancer have been reported in which loss of the derivative chromosome and *VHL* mutations were found in approximately half of the tumors evaluated [15]. Based on these findings, a multistep model for tumorigenesis in these translocation families has been proposed: (a) inheritance of the constitutional chromosome 3 translocation, (b) loss of the derivative chromosome carrying one *VHL* allele, and (c) somatic mutation of the remaining *VHL* allele [14, 16].

1.1.3   VHL Tumor Suppressor Gene: Consequences of Mutational Inactivation in Clear Cell Kidney Tumors

Following the identification of chromosome 3p loss in sporadic clear cell kidney tumors [17], genetic linkage analysis in VHL families [18] led to the positional cloning and identification of the *VHL* tumor suppressor gene on chromosome 3p25 [19]. Biallelic inactivation of *VHL* through loss of the remaining wild-type allele reported in >95% of VHL-associated kidney tumors [20, 21] supports the Knudson "two-hit" model for tumorigenesis involving tumor suppressor genes [22].

The most well-studied function of the VHL protein, pVHL, is its role as the substrate recognition component of an E3 ubiquitin ligase complex consisting of elongins C and B, cullin 2, and Rbx-1 [23–26]. Under normoxic conditions, pVHL binds to the α-subunits of a family of transcription factors known as hypoxia-inducible factors (HIFα) following their hydroxylation on critical proline residues in the HIF oxygen-dependent degradation domain (ODD) by oxygen-dependent HIF prolyl hydroxylases (PHDs), thereby targeting HIFα subunits for ubiquitylation and proteasomal degradation [27, 28]. Under hypoxic conditions, or under normoxia when pVHL is mutated and unable to bind HIFα or other components of the E3 ligase complex, HIFα stabilizes and enters the nucleus where it complexes with HIF-β and transcriptionally activates HIF-target genes [29]. Stabilization of HIF-2α, rather than HIF-1α, appears to be the driving force for *VHL*-deficient kidney tumor development [30, 31], potentially through HIF-2α-specific elevation of c-Myc activity [32]. Upregulation of critical HIF-target genes promotes tumor angiogenesis (*VEGF*, *EPO*), tumor cell growth (*TGF-α*, *PDGF-β*, *Cyclin D*), and glucose metabolism (*GLUT 1*). Inhibition of HIF-target genes has provided great opportunities for molecular targeted therapy for patients with *VHL*-deficient clear cell kidney tumors.

1.1.4   Sporadic Clear Cell Kidney Cancer: Involvement of VHL and PBRM1 Genes

Among sporadic kidney cancer cases, the most common histologic subtype is clear cell, and the majority of these tumors harbor mutations in the *VHL* gene. In fact, in one large case study, the *VHL* gene was inactivated by somatic mutation or promoter hypermethylation in 91% of 205 sporadic clear cell kidney tumors, supporting the hypothesis that *VHL* inactivation is an early event in non-inherited clear cell kidney tumorigenesis [33].

Recent exome sequencing approaches have uncovered a second gene frequently mutated in clear cell kidney tumors. *Polybromo 1* (*PBRM1*), which encodes BAF 180, the chromatin targeting subunit of the PBAF SWI/SNF chromatin remodeling complex [34], was found to be mutated in four of seven clear cell RCC cell lines and 88 of 220 primary clear cell renal tumors [35]. In total, truncating mutations in *PBRM1* were found in 41% of all clear cell tumors and cell lines evaluated, and all

in the context of chromosome 3p loss of heterozygosity where SNP analysis was informative. Since 24 of 38 *PBRM1* mutation-positive cases also had *VHL* mutations, *PBRM1* inactivation may comprise the second major mutational event leading to clear cell kidney tumor development.

1.1.5 Management of VHL-Deficient Kidney Tumors and Therapeutic Considerations

Patients with *VHL*-deficient kidney tumors are managed with active surveillance until the largest tumor reaches 3 cm. At that time, surgical intervention is recommended [36]. Given the multifocality of these tumors and the need for repeated surgical interventions with their attendant morbidity, several centers have attempted to evaluate systemic therapy options in patients with localized VHL-associated tumors. While agents targeting the VEGF and mammalian target of rapamycin (mTOR) pathways are the mainstay of therapy for patients with advanced sporadic clear cell RCC, their utility in VHL patients remains to be determined. In a pilot study of sunitinib in VHL patients, Matin et al. [37] reported responses in 5 of 21 renal tumors in 11 patients, but only infrequent tumor regression in CNS hemangio-blastomas and pancreatic neuroendocrine tumors. Vandetanib, a dual VEGFR and EGFR kinase inhibitor, is currently being studied in patients with VHL-associated renal tumors in a phase 2 study at the National Cancer Institute (NCT00566995). These studies will help determine the tolerability and efficacy of targeting the HIF/VEGF pathway in VHL patients.

1.1.6 Inherited Papillary Kidney Cancer, Type 1: Hereditary Papillary Renal Carcinoma

In contrast to VHL disease, a multisystem disorder, HPRC is a rare, autosomal dominantly inherited disorder in which patients develop bilateral multifocal kidney tumors with papillary type I architecture and no other manifestations (Fig. 1.2a, b) [38]. Type 1 papillary tumors are characterized by delicate papillae with small tumor cells arranged in a single layer containing scant cytoplasm and frequent aggregates of foamy macrophages (Fig. 1.2c). HPRC-associated kidney tumors are characterized by trisomy of chromosomes 7 and 17, and less frequently, chromosomes 12, 16, and 20 [39]. Penetrance has been estimated at 67% by the age of 60 [40]; however, cases of early onset HPRC (i.e., 19 years) have been reported [41]. Less than 50 HPRC families have been described worldwide, underscoring the rarity of this cancer syndrome [42]. Individuals affected with HPRC inherit germline mutations in the *MET* proto-oncogene located on chromosome 7q31 (Fig. 1.2d) [43]. Although hereditary and sporadic papillary type 1 kidney tumors share a distinct morphologic
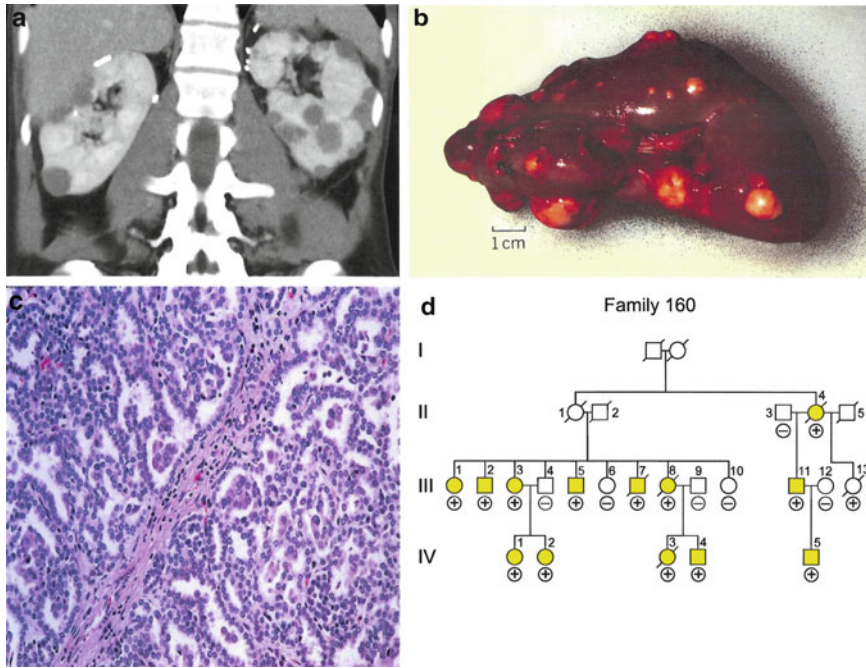
Fig. 1.2 Type 1 papillary kidney cancer in HPRC. Patients with HPRC are at risk for developing bilateral, multifocal kidney tumors (**a** and **b**) with type I papillary histology (**c**). Activating *MET* mutations predispose to kidney cancer in an HPRC kindred (**d**). Adapted from Linehan et al. [127]

phenotype [44], only about 13% of sporadic papillary kidney tumors have been identified with *MET* mutations [45, 46]. The role of MET in the pathogenesis of sporadic papillary kidney cancer remains to be determined.

1.1.7 *MET Proto-oncogene: Consequences of Mutational Activation in HPRC Kidney Tumors*

Linkage analysis in HPRC families with an inherited predisposition to develop bilateral multifocal papillary type 1 kidney tumors led to the identification of *MET* as the responsible gene [43]. To date, all reported *MET* mutations in HPRC families are missense resulting in amino acid substitutions, and all are located in the tyrosine kinase domain of the MET protein. MET is the receptor for hepatocyte growth factor/scatter factor (HGF/SF). HGF ligand binding leads to MET autophosphorylation, followed by phosphorylation of critical tyrosines in the carboxy-terminal docking site, and recruitment of second messenger molecules triggering signaling cascades that drive programs of morphogenesis, mitogenesis, and motogenesis [47, 48].

All of the *MET* mutations in HPRC were shown to activate MET in the absence of HGF ligand, demonstrated oncogenic potential in cell-based assays and in xenograft models [49–51], and were predicted by 3D modeling to stabilize the active kinase conformation [52]. Nonrandom duplication of the chromosome 7 bearing the mutant *MET* allele, which was demonstrated in HPRC kidney tumors [53, 54], may afford a growth advantage to these tumor cells providing a necessary second step in HPRC tumor progression. Inhibition of MET kinase activity offers a promising approach to therapeutic intervention for HPRC patients. Furthermore, the discovery that *MET* has a hypoxia-response element (HRE) in its promoter and was demonstrated to be an HIF-target gene [55] may support the therapeutic use of small molecule HIF inhibitors in targeting tumors in which MET is overexpressed or activated [56].

1.1.8   Management of Papillary Type 1 Kidney Tumors with Activating MET Mutations and Therapeutic Considerations

Kidney tumors in patients with HPRC are managed with active surveillance until the largest tumor reaches the 3 cm threshold. When the largest kidney tumor reaches 3 cm, surgical intervention is recommended [57]. The recent availability of small molecule inhibitors of MET kinase activity has allowed evaluation of MET as a valid therapeutic target in patients with HPRC. Preliminary data from a phase 2 trial of foretinib, a dual MET/VEGFR2 inhibitor, suggest activity in patients with papillary RCC, including those with germline *MET* mutations [58]. Mature data from this trial are awaited and may provide the basis for further evaluation of nonsurgical options in patients with HPRC.

1.1.9   Inherited Chromophobe Kidney Cancer: Birt–Hogg–Dubé Syndrome

BHD syndrome was originally described by three Canadian physicians, for whom the disorder was named, as a rare autosomal dominantly inherited dermatologic disorder in which patients developed hamartomas of the hair follicle (fibrofolliculomas) [59]. Subsequently, kidney cancer families were identified in which fibrofolliculomas cosegregated with bilateral, multifocal kidney neoplasms (Fig. 1.3b, d) [60, 61]. Unlike VHL disease, in which patients only develop clear cell kidney cancer, or HPRC in which papillary type 1 kidney tumors are exclusively found, BHD-associated kidney neoplasms are histologically diverse. BHD syndrome represents the first example of inherited chromophobe kidney cancer, which occurs in about 34% of BHD-associated kidney neoplasia. The most frequent kidney neoplasm
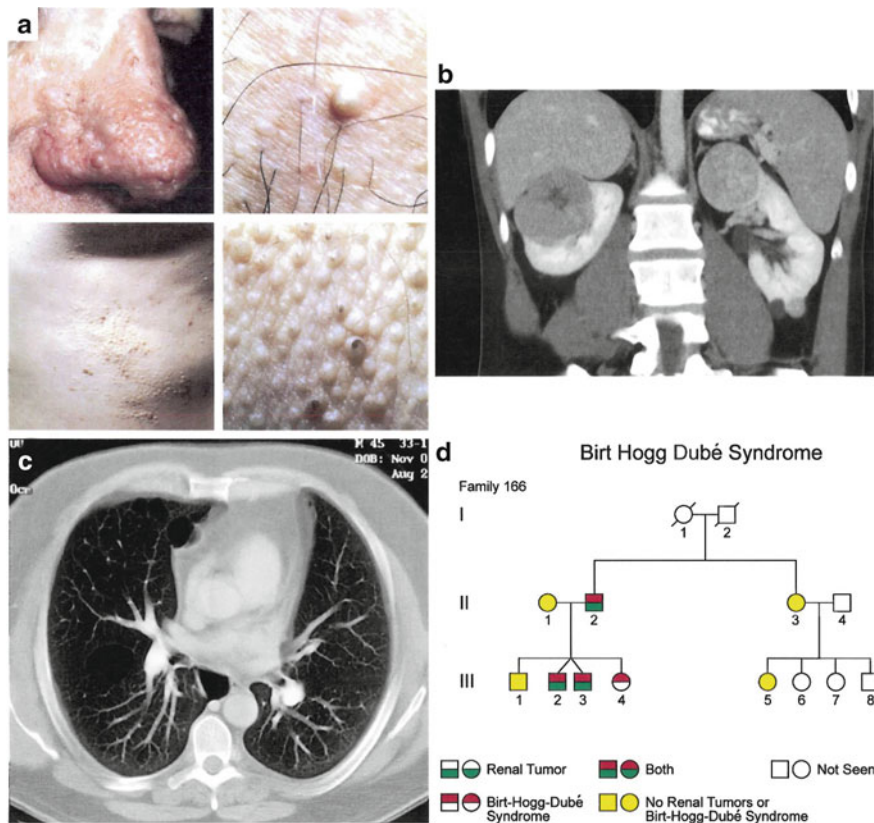
Fig. 1.3 Phenotypic manifestations of BHD syndrome. Individuals affected with BHD are at risk for developing hair follicle tumors called fibrofolliculomas (**a**), bilateral multifocal kidney tumors (**b**), and lung cysts (**c**). Kidney tumors cosegregate with fibrofolliculomas in affected individuals in a BHD kindred (**d**). Adapted from Linehan et al. [127]

found in BHD is the hybrid oncocytic tumor (~50% of cases) with features of chromophobe kidney cancer and oncocytoma, but clear cell tumors (9%) and non-type 1 papillary kidney tumors (<2%) have been described. The average age at diagnosis of kidney neoplasia in BHD patients is 50 years [62].

Individuals who inherit germline mutations in the *FLCN* (*BHD*) gene on chromosome 17p11.2 are at risk for developing the BHD phenotypic manifestations. The phenotypic manifestations of BHD are heterogeneous; some affected individuals in a single family will develop skin lesions, lung cysts with a history of spontaneous pneumothorax or renal neoplasia, or any combination of these phenotypes. Fibrofolliculomas (Fig. 1.3a) are the most highly penetrant manifestations occurring in 82–90% of affected individuals and nearly 100% of BHD families, followed by lung cysts (Fig. 1.3c) that develop in 70–85% of affected individuals, and spontaneous

pneumothorax, which occurs in one-third of BHD patients [63–65]. In two BHD cohorts, approximately 29–34% of affected individuals from almost half of the families presented with kidney neoplasia [64, 65]. However, this high frequency may reflect ascertainment bias as these reports originated from urology clinics. Another cohort of ten BHD families had no kidney neoplasia among the affected individuals [63], whereas a second report of 20 BHD families recruited through a dermatology department indicated kidney neoplasia in only 10% of affected individuals [66]. Early studies had suggested an association between colon polyps and fibrofolliculomas; however, a risk assessment evaluating BHD families seen at the National Institutes of Health did not detect a frequency of colon polyps or cancer that was significantly different from unaffected siblings [61]. Reports from other institutions, however, support colon polyps as part of the phenotype but fall short of statistical significance [67, 68]. Therefore, conclusive evidence for an association of colonic polyps and/or colon cancer with the BHD phenotype will require larger cohort studies.

1.1.10 Folliculin (FLCN) Gene: Consequences of Mutational Inactivation in BHD-Associated Kidney Tumors

Genetic linkage and classical positional cloning methods were used to identify the BHD syndrome locus [69, 70] and subsequently clone the *FLCN* gene located on the short arm of chromosome 17 [71]. The *FLCN* mutation spectrum includes protein truncating mutations (insertion/deletion, nonsense, splice site), infrequent amino acid substitution (missense) mutations [63–66], and large intragenic deletions and duplications [72, 73], but with no convincing genotype–phenotype associations. With the advent of reliable assay systems for intragenic deletions, the *FLCN* mutation detection rate in BHD families is now greater than 90%; however, only rare *FLCN* mutations have been identified in sporadic chromophobe or clear cell kidney tumors [74–76]. Insertion or deletion of a cytosine in a mononucleotide C_8 tract in exon 11 represents a hypermutable site in the *FLCN* gene. The role of *FLCN* as a tumor suppressor gene is supported by the fact that the remaining wild-type *FLCN* allele was inactivated through somatic mutation or chromosome 17p loss in the majority of BHD-associated kidney tumors [77] and that homozygous loss of *Flcn* was confirmed in kidney tumors from *Flcn* knockout mice [78].

In an effort to elucidate FLCN function, interacting protein partners FNIP1 [79] and FNIP2/L [80, 81] were identified. These proteins were found to interact with 5′AMP-activated protein kinase (AMPK), an important energy and nutrient sensor in cells, and a negative regulator of mTOR, the master controller of protein synthesis and cell growth [82]. FLCN through FNIP1 and FNIP2/L may play a role in AMPK-mTOR signaling, an area currently under intense investigation in a number of laboratories. Reports demonstrating mTORC1 [78, 83, 84] and mTORC2 [78] activation in BHD-associated kidney tumors and kidney tumors from *Flcn* knockout mouse models, and conversely, mTOR inactivation in yeast defective for the FLCN homolog

[85] and *Flcn* knockout mouse kidney tumors [86] have led to the hypothesis that the mechanism by which FLCN interacts with and modulates mTOR may be context dependent [87]. Treatment of kidney-directed *Flcn* knockout mouse models with rapamycin, an inhibitor of mTOR, partially reversed the polycystic kidney phenotype suggesting that drugs that target the mTOR pathway (i.e., rapalogues) may provide therapeutic benefit for BHD patients [83, 84].

Emerging data from several labs suggest that FLCN may additionally play a role in TGFβ signaling [88, 89], and that elevated HIF transcriptional activity observed in BHD-associated kidney tumors and *FLCN*-null cell lines results in increased expression of HIF-target genes including glucose transporter 1 (*GLUT1*) and pyruvate dehydrogenase kinase 1 (*PDK1*), and a greater dependency on glucose metabolism for energy ("Warburg effect") [90]. Based on these observations, inhibitors of glucose metabolism may be promising therapeutic approaches to treat kidney tumors arising in the setting of BHD.

1.1.11 Management of FLCN-Deficient Kidney Tumors and Therapeutic Considerations

Kidney tumors in patients affected with BHD syndrome are managed with active surveillance until the largest tumor reaches 3 cm. When the largest kidney tumor reaches 3 cm, surgical intervention is recommended [91]. Preclinical activity of mTOR pathway antagonists in *FLCN*-null models provides the impetus for evaluation of both rapalogues and dual mTORC1/2 inhibitors in BHD patients.

1.1.12 Inherited Papillary Kidney Cancer, Type 2: Hereditary Leiomyomatosis and Renal Cell Carcinoma

This cancer syndrome was first described as an autosomal dominantly inherited dermatologic syndrome known as multiple cutaneous and uterine leiomyomatosis (MCUL) based on the presentation of benign smooth muscle tumors of the skin and uterus but subsequently was renamed hereditary leiomyomatosis and renal cell carcinoma (HLRCC) when an association between the cutaneous and uterine leiomyomas and kidney cancer was confirmed in these kindreds (Fig. 1.4d) [92]. Leiomyomas of the skin and uterus are the most common manifestations of HLRCC occurring in 76–100% of affected individuals and nearly 100% of affected women, respectively (Fig. 1.4b, c) [93–95]. HLRCC patients are at risk for developing a highly aggressive form of kidney tumor displaying unique papillary architecture (papillary type 2) and histologic features characterized by large nuclei containing inclusion-like, orangiophilic nucleoli with a perinucleolar halo [96]. Kidney tumors present in about 15% of affected individuals (Fig. 1.4a), are often solitary and unilateral with
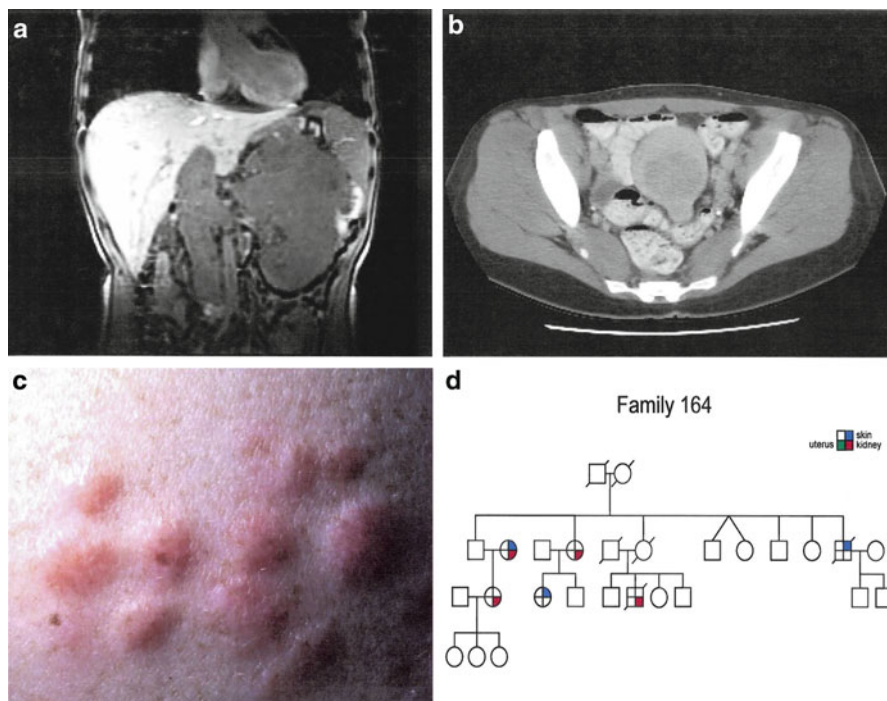
Fig. 1.4 Phenotypic manifestations of HLRCC. Patients with HLRCC are at risk for developing highly aggressive type 2 papillary kidney tumors (**a**), uterine leiomyomas (**b**), and multiple cutaneous leiomyomas (**c**). ×4 magnification. Phenotypic heterogeneity is a feature of HLRCC (**d**). Adapted from Linehan et al. [127]

early age of onset (as young as 18 years), and can metastasize and cause death within 5 years of diagnosis [94]. Collecting duct carcinoma has also been reported in association with HLRCC [94, 97]. This inherited cancer syndrome is rare with fewer than 150 families reported worldwide [98].

1.1.13 Fumarate Hydratase (FH) Gene: Consequences of Mutational Inactivation in HLRCC Kidney Cancer

The disease locus for HLRCC was mapped to chromosome 1q42–44 by genetic linkage analysis in HLRCC families [92], and the predisposing gene was identified as the *FH* gene, which encodes the Krebs cycle enzyme fumarate hydratase (FH) and catalyzes the conversion of fumarate to malate [99]. Germline mutations in *FH* including protein truncating mutations, missense mutations resulting in amino acid substitutions, and whole or partial gene deletions have been

identified in nearly 90% of individuals affected with HLRCC [94, 95, 99] and result in reduced or undetectable fumarate hydratase activity [97, 99, 100]. Biallelic inactivation of *FH* has been detected in nearly all HLRCC-associated tumors (skin and uterine leiomyomata, kidney tumors) underscoring a tumor suppressor role for *FH* [92, 97, 99]. Of note, only a very low frequency of *FH* mutations has been found in sporadic counterpart uterine fibroids and kidney tumors [101].

Pseudohypoxic drive is generally thought to be the driving force for tumors that develop in the setting of HLRCC kidney cancer [102]. Mutational inactivation of *FH* leads to an accumulation of fumarate, which has been shown to inhibit HIF PHD by competitive inhibition of its cosubstrate α-ketoglutarate [103, 104]. Without hydroxylation of critical prolines in HIFα, pVHL cannot bind to and target HIFα for ubiquitin-mediated degradation by the E3 ubiquitin ligase complex. HIFα stabilization enables transcriptional activation of HIF-target genes that support glucose uptake (*GLUT1*) and tumor angiogenesis (*VEGF*, *EPO*) and proliferation (*TGF-α*, *PDGFβ*), accounting for the highly aggressive nature and early onset of *FH*-deficient kidney tumors. Further studies using a renal tumor cell line established from an *FH*-deficient tumor have provided additional insight into the mechanism of metabolic regulation of HIFα stabilization in HLRCC tumors. Findings from these studies suggest that *FH*-deficient tumor cells undergo a "glycolytic switch" in response to HIFα stabilization (a direct consequence of fumarate accumulation) resulting in an increase in glucose uptake and elevated reactive oxygen species (ROS) production, possibly through PKC-δ-mediated activation of NADPH oxidase [105]. Generation of ROS may drive HIFα stabilization and glucose "addiction" by depleting cellular stores of Fe^{+2}, another cofactor of PDH, further blocking HIFα prolyl hydroxylation. Targeting ROS may be one promising approach to therapy against *FH*-deficient HLRCC kidney cancer. Additional experiments in *FH*-deficient mouse embryonic fibroblasts support the development of an early, progressive metabolic profile that supports and, indeed, drives tumorigenesis in HLRCC kidney tumors mirroring the metabolic alterations that produce the "Warburg effect" [106]. Targeting these aberrant metabolic pathways may be important for therapeutic treatment of HLRCC kidney tumors.

1.1.14   Management of FH-Deficient Kidney Tumors and Therapeutic Considerations

Kidney tumors in patients with HLRCC can be extremely aggressive and can spread when the tumors are very small. Active surveillance of HLRCC-associated kidney cancer is not recommended. When a kidney tumor is detected in a patient affected with HLRCC, early surgical management is recommended [107]. Elevated HIF transcriptional activity in HLRCC-associated tumors and a heightened dependence on glucose has led investigators to propose that strategies targeting the HIF pathway may be effective in this population. This hypothesis is being tested in an ongoing

phase 2 study of a combination of bevacizumab (monoclonal antibody against VEGF-A) and erlotinib (oral kinase inhibitor of EGFR) in patients with advanced HLRCC-associated kidney tumors (NCT01130519).

1.1.15   Inherited Kidney Cancer Associated with Succinate Dehydrogenase Subunit Mutations: SDHB/D Mutation-Associated Familial Kidney Cancer

Inherited mutations in subunits of another Krebs cycle enzyme, SDH, have been identified in the germline of individuals with head and neck paragangliomas (HPGL) and/or adrenal or extra-adrenal pheochromocytomas [108, 109]. Confirmation that kidney cancer was part of the HPGL phenotype came when three individuals from two kindreds with inherited paragangliomas and germline *SDHB* mutations were diagnosed with early onset clear cell kidney tumors [110]. Subsequently, other histologic subtypes were identified in individuals with *SDHB* mutations including papillary type 2, oncocytoma, and chromophobe kidney tumors [111–113]. The age of diagnosis is relatively early with a mean age of 34 years (range 10–62 years) [113]. For diagnostic purposes, it is also important to note that germline *SDHB* mutations can be found in individuals with familial kidney cancer but without personal or family history of paragangliomas or pheochromocytomas [112]. One case of kidney cancer in association with a germline *SDHD* mutation has been reported [114].

The *SDHB/D* mutation spectrum associated with early onset kidney cancer includes missense and protein truncating (nonsense and frameshift) mutations [110–113]. No association of *SDHB/D* mutations with sporadic kidney tumors was observed [110]. Somatic loss of the wild-type *SDHB* allele was detected in several SDH-associated familial kidney tumors, suggesting a "two-hit" tumorigenesis model for SDH-associated kidney cancer and a tumor suppressor role for SDH [110].

1.1.16   SDHB/D Genes: Consequences of Mutational Inactivation in SDH-Associated Familial Kidney Tumors

Analogous to *FH*-deficient kidney tumors, mutational inactivation of *SDHB/D* abrogates SDH activity leading to a blockade of the Krebs cycle and accumulation of succinate, which inhibits HIF PHD through competition with its cosubstrate, α-ketoglutarate. Through this "pseudohypoxic" HIFα stabilization, transcriptional activation of HIF-target genes (i.e., VEGF, PDGFβ) may drive tumorigenesis in *SDH*-deficient kidney tumors [103, 104, 115]. Targeting these genes may offer opportunities for directed therapy for SDH-associated kidney cancer patients.

1.1.17   Management of SDH-Deficient Kidney Tumors

There is less experience with clinical management of kidney cancers associated with germline mutation of the *SDH subunit* genes. However, these tumors can be very aggressive, and until further clinical experience is gained, early surgical intervention of SDH-associated kidney cancers is also recommended.

1.1.18   Familial Hamartoma Syndromes Associated with Kidney Cancer: Tuberous Sclerosis Complex

TSC is an autosomal dominantly inherited multisystem disorder in which affected individuals are at risk for developing facial angiofibromas, which are the most penetrant manifestations, benign renal angiomyolipomas comprising abnormal blood vessels, smooth -muscle, and fat cells, and pulmonary lymphangiomyomatosis that can develop in 26–39% of adolescent girls and women. Additionally, over 80% of patients develop cerebral cortical tubers that can lead to mental retardation, autism, and epilepsy, which occur in 70–80% of patients [116].

In addition to bilateral multifocal angiomyolipomas, kidney cancer occurs in TSC patients at about the same frequency as the general population, with a life time risk of 2–3% [116], but with earlier onset (average age, 28 years) [117]. Variable renal tumor histologies have been reported among TSC patients including clear cell, papillary, and chromophobe renal tumors and oncocytomas [118, 119].

1.1.19   TSC1/TSC2 Tumor Suppressor Genes: Consequences of Mutational Inactivation in TSC and Therapeutic Implications

Classic linkage analysis in large TSC families and positional cloning enabled the mapping of the genes responsible for the manifestations of TSC: *TSC1* on chromosome 9q34 encoding hamartin [120] and *TSC2* located on chromosome 16p13 that encodes tuberin [121]. Somatic mutation of the remaining wild-type *TSC1* or *TSC2* allele or loss of chromosomal sequences was identified in TSC-associated lesions (angiomyolipomas, lymphangiomyomata) confirming that *TSC1* and *TSC2* act as tumor suppressor genes [122, 123].

TSC1 and TSC2 proteins form a complex that negatively regulates mTOR in response to 5′-AMPK activation through GTP hydrolysis of the small GTPase Rheb [124, 125]. Mutational inactivation of either of these proteins leads to upregulation of mTOR activity and uncontrolled cell growth. Treatment of TSC patients with mTOR inhibitors (i.e., sirolimus) has proven partially successful in reducing angiomyolipomas and lymphangiomyomatosis [126].
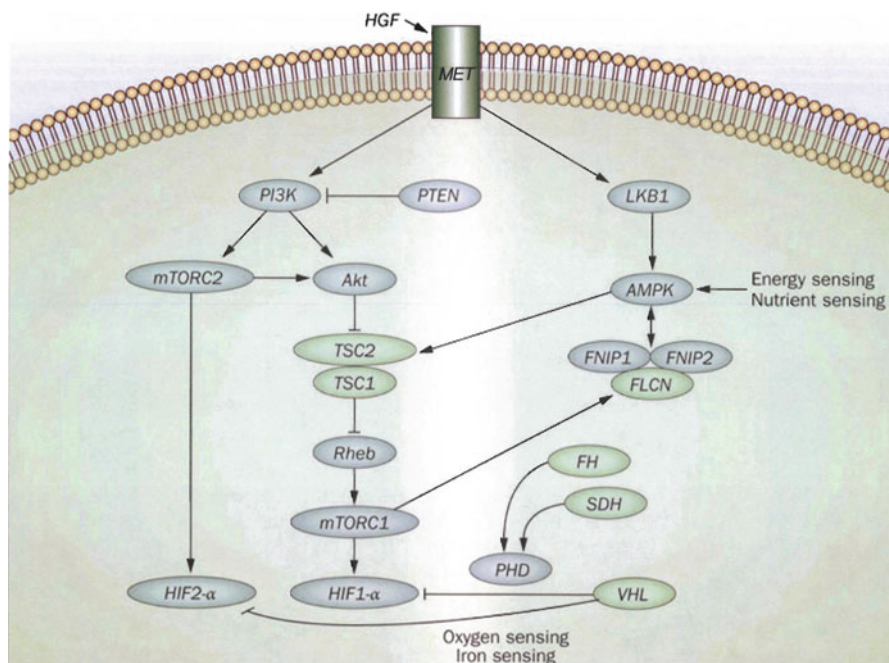
Fig. 1.5 The genetic basis for kidney cancer. The seven kidney cancer predisposing genes—*VHL*, *MET*, *FLCN*, *FH*, *SDH*, *TSC1*, and *TSC2*—interact in common pathways that serve as oxygen, nutrient, and energy sensors in cells. Dysregulation of these pathways through mutation of the cancer predisposing genes promotes tumorigenesis. Targeting these pathways provides opportunities for directed therapies for kidney cancer patients. Used with permission [3]

1.2   Summary

Inherited kidney cancer is not a single entity but a group of distinct tumor subtypes classified by their histologies and caused by mutations in seven predisposing kidney cancer genes—*VHL*, *MET*, *FLCN*, *FH*, *SDH*, *TSC1*, and *TSC2*—that cause six well-described inherited kidney cancer syndromes—VHL, HPRC, BHD, HLRCC, SDH-RCC, and TSC. These kidney cancer genes interact through common pathways that are important for oxygen, nutrient, and energy sensing in cells (Fig. 1.5), and that predispose to malignancy when dysregulated. Studies of families with inherited kidney cancer syndromes have provided insight into the molecular mechanisms responsible for tumorigenesis and formed the basis for development of targeted therapies to treat patients with these syndromes and, indeed, for treatment of patients with the more common forms of sporadic kidney cancer.

References

1. Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. CA Cancer J Clin 60:277–300
2. Linehan WM, Rini B, Yang JC (2010) Cancer of the kidney. In: DeVita VT, Lawrence TS, Rosenberg SA (eds) Cancer: principles and practice of oncology, 9th edn. Lippincott Williams & Wilkins, Philadelphia, PA, pp 1161–1182
3. Linehan WM, Srinivasan R, Schmidt LS (2010) The genetic basis of kidney cancer: a metabolic disease. Nat Rev Urol 7:277–285
4. Lonser RR, Glenn GM, Walther MM, Chew EY, Libutti SK, Linehan WM et al (2003) von Hippel-Lindau disease. Lancet 361:2059–2067
5. Maher ER (2004) von Hippel-Lindau disease. Curr Mol Med 4:833–842
6. Stolle C, Glenn GM, Zbar B, Humphrey JS, Choyke P, Walther MM et al (1998) Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene. Hum Mutat 12:417–423
7. Chen F, Kishida T, Yao M, Hustad T, Glavac D, Dean M et al (1995) Germline mutations in the von Hippel-Lindau disease tumor suppressor gene: correlations with phenotype. Hum Mutat 5:66–75
8. Crossey P, Eng C, Ginalska-Malinowska M, Lennard T, Wheeler D, Ponder B et al (1995) Molecular genetic diagnosis of von Hippel-Lindau disease in familial pheochromocytoma. J Med Genet 32:885–886
9. Stebbins CE, Kaelin WG Jr, Pavletich NP (1999) Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function. Science 284:455–461
10. Clifford SC, Cockman ME, Smallwood AC, Mole DR, Woodward ER, Maxwell PH, Ratcliffe PJ, Maher ER (2001) Contrasting effects on HIF-1alpha regulation by disease-causing pVHL mutations correlate with patterns of tumourigenesis in von Hippel-Lindau disease. Hum Mol Genet 10:1029–1038
11. Hoffman MA, Ohh M, Yang H, Klco JM, Ivan M, Kaelin WG Jr (2001) von Hippel-Lindau protein mutants linked to type 2C VHL disease preserve the ability to downregulate HIF. Hum Mol Genet 10:1019–1027
12. Maranchie JK, Afonso A, Albert PS, Kalyandrug S, Phillips JL, Zhou S et al (2004) Solid renal tumor severity in von Hippel Lindau disease is related to germline deletion length and location. Hum Mutat 23:40–46
13. Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC et al (1979) Hereditary renal-cell carcinoma associated with a chromosomal translocation. N Engl J Med 301:592–595
14. Schmidt L, Li F, Brown RS, Berg S, Chen F, Wei MH et al (1995) Mechanism of tumorigenesis of renal carcinomas associated with the constitutional chromosome 3;8 translocation. Cancer J Sci Am 1:191–195
15. Bonné AC, Bodmer D, Schoenmakers EFPM, van Ravenswaaij CM, Hoogerbrugge N, van Kessel AG (2004) Chromosome 3 translocations and familial renal cell cancer. Curr Mol Med 4:849–854
16. Bodmer D, Eleveld MJ, Ligtenberg MJ, Weterman MA, Janssen BA, Smeets DF et al (1998) An alternative route for multistep tumorigenesis in a novel case of hereditary renal cell cancer and a t(2;3)(q35;q21) chromosome translocation. Am J Hum Genet 62:1475–1483
17. Zbar B, Brauch H, Talmadge C, Linehan WM (1987) Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature 327:721–724