Molecular Pathogenetics of Renal Cancer

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Abstract

Recent developments in genetics and molecular biology have led to an increased understanding of the pathobiology of renal cancer. Thorough knowledge of the molecular pathways associated with renal cancer is a prerequisite for novel potential therapeutic interventions. Studies are ongoing to evaluate novel anticancer agents that target specific molecular entities. This article reviews current knowledge on the genetics and molecular pathogenesis of sporadic and inherited forms of renal cancer.

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Introduction

Renal cancer is comprised of several different types of cancer, each with a different histology, clinical course, and genetic background (table 1) [1]. For these different tumor types, hereditary, familial and sporadic forms exist. Most of our current knowledge was originally derived from studies on familial forms of the disease, but subsequent studies of sporadic cancers found similar changes (table 2) [2].

Renal cell carcinoma (RCC) is increasing in frequency in the United States and is often detected late in the course

of disease due to nonspecific symptoms [3]. Understanding of the molecular alterations in patients with familial and sporadic renal tumors might provide some insight into the underlying mechanisms of RCC initiation and progression, which will be reviewed separately for sporadic and inherited forms.

Sporadic RCC

Clear-Cell RCCs

The von-Hippel-Lindau (VHL) Tumor Suppressor Gene

Clear-cell RCCs are characterized by a non-homologous chromatid exchange leading to loss of genetic material from the short (p) arm of chromosome 3 and gains of material to the long (q) arm of chromosome 5 [4]. Loss of heterozygosity (LOH) studies in sporadic RCCs identified three common regions of allelic loss: 3p12–14 [5], 3p21–22 [6], and 3p25–26 [7].

Loss of 3p and gain of 5q implicate genes within these regions as being involved in clear-cell RCC tumorigenesis. Tumor-suppressor genes are likely to be found in areas of chromosomal loss (3p), while oncogenes are usually found in areas of chromosomal gain (5q). The only gene definitively shown to be involved in the development of such tumors is the tumor suppressor von-Hippel-Lindau (VHL) gene, located at 3p25 [7, 8]. Renal cell can-

Table 1. Cytogenetic features of RCC

Tumor	Site of origin	Genes	Numerical chromosomal abnormalities	Chromosomal translocations
Clear-cell RCC	Proximal renal tubule	VHL, BHD, RASSF1A, FHIT	-3p, +5q, -Y, -8p, -9p, -14q	t(3;5)(p;q)
Papillary RCC	Proximal renal tubule	c-MET, FH, TFE3, PRCC, RCC 17	+7, +17, -Y, +12, +16, +20	t(X;1)(p11.2;q21.2) t(X;17)(p11.2;q25.3)
Chromophobe RCC	Intercalated cell of renal collecting duct	BHD	-1, -2, -6, -10, -13, -17, -21	Unknown
Collecting duct RCC	Renal collecting duct	Unknown	-1q32, -6p, -8p, -21q	Unknown

Table 2. Inherited forms of RCC

	Gene	Location	Protein	Function	Histology
Von Hippel-Lindau disease	VHL	3p25	pVHL	Tumor suppressor	Clear cell RCC
Hereditary papillary renal cancer	cMET	7q31	Hepatocyte growth factor receptor	Oncogene	Type-1 papillary RCC
Hereditary leiomyomatosis renal cancer	FH	1q42-43	Fumarate hydratase	Tumor suppressor	Type-2 papillary RCC
Birt-Hogg-Dubé syndrome	BHD	17p11.2	Folliculin	Tumor suppressor	Chromophobe RCC, oncocytoma

cers found in VHL follow Knudson's theory of the two-hit model of carcinogenesis [7]. The VHL gene is inactivated in patients with VHL and in almost 100% of sporadic clear-cell RCC [8, 9].

To evaluate whether inactivation of the VHL gene is essential for tumor formation, the patterns and extent of allelic losses on 3p were compared in a set of conventional RCCs with and without VHL mutations. Although the frequency of 3p12-22 LOH was similar in both tumor types, LOH at 3p25-26 was significantly less frequent than LOH at 3p12-22 in tumors without VHL mutation, whereas LOH at 3p25-26 and 3p12-22 was similar in tumors with or without VHL mutation [10-12]. These results support the presence of both VHL-dependent and VHL-independent carcinogenic pathways, and indicate that inactivation of a tumor-suppressor gene at 3p12-22 might play an important role in both these pathways.

The inactivation of VHL is possible by mutation, deletion, or methylation. Mutations of the VHL gene seen in sporadic RCC differ from those seen in RCC associated with VHL disease. In sporadic RCC, 45% of the mutations are clustered in the second exon, although abnormalities are seen in all three exons. Large deletions are not observed, and 48% of the mutations are micro-deletions or insertions, resulting in frame shifts of the protein-coding sequence [9]. The role of the VHL protein in carcinogenesis will be discussed in the section of inherited RCC.

Other Tumor Candidate Genes in the 3p Region

Apart from the locus in 3p25-26, other loci on the 3p chromosome, such as 3p12, 3p14.2, 3p21.1, 3p21.3, 3p22 and 3p26.2, might be involved in clear-cell RCC initiation or progression, and tumor-suppressor genes at these loci are inactivated. Non-papillary renal carcinoma-1 (NRC-1) and non-papillary renal carcinoma-2 (NRC-2) genes located in 3p12 and 3p14.2 regions, respectively, have also been related to RCC [13, 14]. Within the NRC-2 region, the tumor-suppressive region was further narrowed down to a genomic segment which contains both exons 9 and 10 of the fragile histidine triad gene (FHIT) [14].

Tumor-suppressive activity within the NRC-1 region was also observed in VHL-defective RCC cell lines, and it was hypothesized that NRC-1 might act either downstream from VHL within the same pathway or act in a pathway not related to VHL [15]. Moreover, a function in apoptosis has been suggested, but the exact role of NRC-1 in RCC awaits the identification of further candidate genes in this region.

The 'down-regulated in RCC' gene (DRR1) has been identified in the chromosomal 3p21.1 region. A reduced expression of this gene in clear-cell RCCs, and growth suppression after transfection of DRR1-negative cell lines with wild-type DRR1, support a role for this gene in RCC development [16].

Hypermethylation and subsequent silencing of RASSF1A, a tumor-suppressor gene located in the 3p21.3 region, has been demonstrated in clear-cell RCCs [17]. RASSF1A shows significant homology to the mouse RAS protein Nore1 and to the rat protein Maxp1, both interacting with RAS in a GTP-dependent manner [18]. The presence of conserved domains in the RASSF1A protein, known to play important roles in several signal transduction pathways, supports a role for RASSF1A in RAS signaling [18]. As such, inactivation of the RASSF1A gene might have the same cellular effect as oncogenic RAS mutations [18].

Transforming growth factor- β (TGF- β) is a potent inhibitor of epithelial cell growth. By binding to the TGF- β receptor complex, consisting of type I (T β R-I) and type II (T β R-II) receptors, its biological activity is exerted. The gene for the T β R-II has been mapped to chromosome 3p22. Alterations in T β R-II make epithelial tumors insensitive to the inhibitory effects of TGF- β and are correlated with malignant progression [19]. Rescue of wild-type T β R-II activity by gene transfection restored TGF- β responsiveness and reduced the growth rate in different subtypes of human RCC and in a mouse RCC cell line [20, 21], suggesting a role for T β R-II and the TGF- β -regulated pathway in RCC development [19]. Further support for such a role was demonstrated through altered expression of proteins downstream in the TGF- β -signaling pathway, such as SMAD2 and SMAD4 [22]. Significant elevations of plasma TGF- β levels have been recorded in patients with RCC [23].

Another candidate gene, located on 3p26.2, is the 8-oxoguanine DNA glycosylase gene (OGG1) involved in DNA repair. LOH for this gene was found in 85% of conventional RCCs, while an inactivating mutation was also reported [24].

Based on the frequent loss of chromosome 3 sequences in conventional RCCs, the chromosome 3 candidate genes discussed above are suggested to be specific for this subtype of RCC. However, silencing of the RASSF1A gene by promoter hypermethylation has also been detected in papillary RCCs, and genetic alterations in the T β R-II gene have been found in other RCC subtypes [25]. In addition, VHL and FHIT locus loss was demonstrated in all RCC subtypes, but differed in pattern and prognostic significance [25]. Thus, there are overlaps in these carcinogenic pathways between the different RCC subtypes.

Tumor Candidate Genes in Other Regions

No specific proto-oncogenes from 5q have been found mutated in clear-cell RCC [26]. With advancing grade

and stage, clear-cell RCCs exhibit the loss of a variety of stereotypical chromosomal regions, most commonly in 8p, 9p, 14q, 6q and 17p chromosomes [27].

RCC rarely acquires mutations in the p53 tumor-suppressor gene, suggesting that p53 signaling might be repressed by some other mechanism [28]. In RCC cells, p53 undergoes all expected conversions in response to DNA damage but paradoxically does not induce transactivation [28]. This situation can be explained either by a lack of some p53 counterpart that is essential for transactivation or by the presence of an inhibitor of transactivation [28]. There is a detectable repression of p53 transactivation in normal kidney cells that is also dominant in cell fusion experiments, indicating that RCC cells may use an existing kidney-specific mechanism of p53 attenuation to achieve complete inhibition of the p53 pathway [28].

Abnormalities of other tumor-suppressor genes, such as the RB gene, MCC and APC genes, WT1 gene and nm23 gene, have been implicated in sporadic RCC [29].

Oncogenes and Growth Factors in Sporadic RCC

A number of oncogenes have been evaluated in sporadic RCCs. The ras family has been extensively evaluated but does not appear to be critical in RCC [30]. Conflicting results have been reported concerning the expression of HER-2/neu in RCC with most investigators showing a low expression [31]. Abnormal expression of the myc and fas oncogenes has also been reported in renal carcinomas with significant elevation in 50–90% of sporadic renal cancers [32, 33]. Other oncogenes, such as abl, fes, fms, myb, src, and raf, are not expressed or are expressed infrequently in RCC [33].

Activation of several growth factors might be important in the pathogenesis of RCC. Epidermal growth factor (EGF), a potent mitogen, stimulates cultures of RCC cell lines [34]. However, most investigators have not detected EGF in tumor tissue from patients with RCC [35]. Overexpression of the EGF receptor has been reported by several authors [35–37]. Elevated levels of transforming growth factor- α (TGF- α) have been observed in VHL-deficient cancer cells [38]. TGF- α acts as growth-stimulating factor and induces the expression of the EGF receptor. Blockage of this receptor by a monoclonal antibody inhibits the growth of RCC cell lines cultured with TGF- α [36]. TGF- α acts synergistically with TGF- β in inducing transformation and, as such, they are both involved in RCC-related pathways [38].

Other growth factors might also play a role in the initiation or progression of RCC. Truncated fibronectin is secreted by the ACHN RCC cell line and stimulates DNA

synthesis in the absence of other growth factors [39]. Hepatocyte growth factor appears to stimulate proliferation in some RCC cell lines [40]. Other RCC cell lines appear to secrete an autocrine growth promoter when cultured in the presence of 5-fluorouracil [41]. This growth factor potentiated the effects of EGF, TGF- β , and other mitogens. Sera from patients with end-stage renal disease stimulate proliferation of RCC cell lines, and the uncharacterized growth factor implicated in this proliferation does not appear to be EGF, basic fibroblast growth factor (bFGF), or platelet-derived growth factor (PDGF) [42].

Papillary RCC

Papillary RCCs (PRCCs) are suggested to be of renal proximal tubular origin and two histological subtypes exist, classified as type I and type II [43, 44]. Type-I PRCC is less frequent, it is associated with c-MET mutations, and is seen in the hereditary papillary renal cancer syndrome and occasional sporadic PRCC (with or without MET mutations) [44]. Type-II PRCC does not harbor c-MET mutations and is the type most commonly encountered sporadically [45].

A combination of gains of chromosomes 7 and 17 and loss of the Y have been found in PRCCs [46–48]. A relative gain of chromosome 3, 3q material, or both has occasionally been found, although not in the setting of detectable VHL gene mutations [46–48]. There are also reports regarding gains at chromosomes 16 and 20, especially in advanced stages of PRCC [46–48] and chromosomal losses of 1p, 6q, 9p [46, 47].

A rare subset of PRCCs contains losses of the short arm of the X chromosome that are associated with translocations to various chromosomes including T(X;1)(p11;q21), which have repeatedly been encountered [49]. Positional cloning of the translocation breakpoint revealed an in-frame fusion of the TFE3 gene on the X chromosome to a novel gene, PRCC, on chromosome 1 [49, 50]. TFE3 is a ubiquitously expressed transcription factor belonging to the family of E-box binding factors [51].

The PRCC protein is an ubiquitously expressed nuclear protein characterized by a relatively high proline content, without homology to any other known protein [50, 52]. The in-frame fusion results in two fusion genes, the TFE3PRCC and the PRCCTFE3, which are both expressed in t(X;1)-positive tumor cells [50]. The fusion protein PRCCTFE3 retains all the domains of TFE3 necessary for transcriptional activation. The PRCC protein interacts with MAD2B, a human homologue of the yeast

MAD-family protein Mad2, a component of the mitotic spindle checkpoint [53]. Mitotic checkpoint proteins have been shown to be essential for delaying the onset of anaphase and thus preventing mis-segregation of chromosomes during metaphase [54, 55]. Recently, the MAD2B protein has been found to bind indirectly to the anaphase-promoting complex (APC) [56] and to act as an inhibitor of Cdh1-mediated activation of APC [54, 57]. There is evidence suggesting that expression of PRCC/TFE3 leads to impairment of mitotic checkpoint control through interference with MAD2B binding and thus, in a dominant-negative fashion, to papillary RCC development [25].

Cytogenetic studies revealed variant translocations in which TFE3 is fused to other genes such as RCC17 on chromosome 17, PSF on chromosome 1, and NonO (p54^{nrb}) on chromosome X [49, 58]. As PSF and NonO (p54^{nrb}) both encode pre-mRNA splicing factors, a similar role was suggested for PRCC [59]. Although activating mutations of the MET proto-oncogene, which encodes the hepatocyte growth factor receptor, are common in families with hereditary papillary renal cancer, only a few sporadic PRCCs are found to have MET mutations [60]. Nevertheless, two- to threefold gains in chromosome 7 or the 7q31 location of the MET proto-oncogene have been demonstrated in sporadic PRCC [48, 61]. Gains of chromosome 7q harboring a wild-type MET may be carcinogenic by virtue of increased gene copy number and perhaps increased cellular responsiveness to hepatocyte growth factor. The relative paucity of MET mutations found in sporadic papillary tumors may be explained by the fact that gains of wild-type MET copy number may be sufficient for tumorigenesis [61].

Chromophobe RCC

Chromophobe RCCs (CRCCs) arise from the intercalated cells of the cortical collecting ducts [62]. Cytogenetically, the CRCC shows widespread LOH of chromosomes 1, 2, 6, 10, 13, 17 and 21 and hypodiploidy due to non-random multiple chromosome losses [63]. The loss of chromosome Y in tumors from male patients has also been recorded [43]. Allelic changes in 3p have also been described, but these results could be explained by the tendency for loss of the entire chromosomes of these specific RCCs [64]. These multiple losses have made it challenging to define specific genes essential to the development of CRCC.

The p53 tumor-suppressor gene has been implicated based on the common finding of chromosome 17 loss, 17p LOH, and the detection of p53 mutations in 30% of these tumors [65]. The recently cloned gene for the Birt-Hogg-Dubé syndrome may shed light on CRCC, because CRCCs have been found at increased frequency in patients with this syndrome [66].

Collecting Duct Carcinoma

The collecting duct carcinoma is a rare tumor which is derived from the collecting duct epithelia [62]. Data regarding the cytogenetic abnormalities of collecting duct carcinoma are scarce. Conflicting findings have been reported, with some studies detecting monosomies and others reporting trisomies.

Three collecting duct cancers have been characterized cytogenetically. Each cancer was consistently monosomic for chromosomes 1, 6, 14, 15, and 22 [67]. LOH at 1q32.1–32.2, 6p, 8p, and 21q was subsequently reported in a larger series [67–69]. Gains at chromosomes 16 and 20 and losses at 1, 2, 9, 11, and 18 have also been revealed [68]. Trisomy 17, trisomy or tetrasomy 7, or deletion of chromosome 3p were not observed by cytogenetic analysis, differentiating these tumors from papillary and clear-cell RCC, respectively [69].

Inherited RCC Biochemical Pathways

There are four types of inherited epithelial kidney cancer in which the responsible genes have been identified (table 2): (a) VHL clear-cell RCC, (b) hereditary papillary renal carcinoma (HPRC), which mainly refers to type-1 papillary renal carcinoma, (c) hereditary leiomyomatosis RCC (HLRCC), where type-2 papillary renal carcinoma and other carcinomas are included, and (d) the Birt-Hogg-Dubé (BHD) syndrome, where chromophobe renal carcinoma and oncocytoma predominate.

VHL Clear-Cell RCC

Patients with VHL disease are at significant risk for the development of early onset, bilateral, multi-focal renal tumors, and renal cysts [70]. Genetic linkage analysis of the VHL families revealed the VHL tumor-suppressor gene on the short arm of chromosome 3 at the 3p25 region [70, 71]. In hereditary cancers, patients inherit one defec-

Fig. 1. The two ‘hit’ hypothesis of carcinogenesis in VHL disease. **a** Patients inherit a mutated VHL allele as a first ‘hit’ (black bar). **b** In kidney cells a balanced chromosomal translocation occurs. **c** Loss of heterozygosity (LOH) by losing one paternal allele is the second ‘hit’ of carcinogenesis.

tive tumor-suppressor gene from one parent in every cell (one ‘hit’) but no tumors occur until their remaining allele becomes mutated or deleted (i.e. somatic loss, a second ‘hit’, the Knudsen hypothesis; fig. 1). This two-hit hypothesis is supported by the finding that all patients with VHL syndrome have been found to harbor a germline VHL gene that is either mutant or partially or completely deleted [72]. Cells from the clear-cell RCCs of VHL patients have also lost their normal unaffected copy of VHL [73]. Because patients with familial cancers such as VHL clear-cell RCC inherit a germline mutation, they only require one hit to inactivate the remaining tumor-suppressor gene. Patients with sporadic tumors require two hits, which occur less frequently [74].

VHL syndrome demonstrates considerable phenotypic diversity and not all germline VHL mutations are associated with a risk of RCC development [70]. There is a marked genotype–phenotype correlation between VHL gene mutations and manifestations of the different phenotypes. Based on these differences, VHL families have been divided into two major subgroups: VHL types 1 and 2 [75, 76]. VHL type 1 represents the most common group, and is characterized by a predisposition to develop retinal angiomas, central nervous hemangioblastomas, and conventional RCCs. VHL type 2 is subdivided into type 2A and 2B. VHL type 2A is the next most common

Fig. 2. Hypoxia-inducible pathways. Signaling starts with the interaction of growth factors with several tyrosine kinase cell surface receptors. The phosphatidylinositol 4,5-bisphosphate-AKT-mammalian target of the rapamycin pathway (PI3K-AKT-mTOR) and the Ras/Raf/MEK/mitogen-activated protein kinase (MAPK) pathway are activated. VHL protein complexes with elongin B, elongin C, Cullin2, and Rbx1 and forms a ubiquitin ligase. In the presence of oxygen, VHL recognizes hydroxylated proline residues on HIF- α and binds them. Once bound, HIF becomes ubiquitinated and tagged for proteasome-dependent protein degradation. In the absence of oxygen or with loss of pVHL function, HIF- α is allowed to accumulate, ultimately binding to HIF- β and resulting in increased expression of hypoxia-inducible genes. The later encode proteins essential to cancer cell functions under hypoxia, such as glucose transport, angiogenesis, glycolysis, cell growth, migration and pH control. Inhibition of this pathway by small molecules targeted against various steps should at least partly reverse the molecular consequences of VHL loss of function and result in anticancer effects (shown as grey-shaded arrows and boxes). EGFR = Epidermal growth factor receptor; VEGFR = vascular endothelial growth factor receptor; IGFR = insulin growth factor receptor; MEK = mitogen-activated protein (extracellular signal regulated kinase); HIF = hypoxia-inducible factor; FIT = farnesyl transferase inhibitors; VBC = ubiquitin ligase complex; Hsp90 = heat shock protein-90; TGF = transforming growth factor; CAIX = carbonic anhydrase IX; CXCR4 = chemokine receptor 4; Glut-1 = glucose transporter-1.

form and is characterized by a predisposition to develop pheochromocytomas in addition to the manifestations of VHL type 1. The rarest form of VHL is type 2B, which is characterized by a predisposition to develop pheochromocytomas with little or no tendency to develop RCCs [75, 76]. The genotype may predict the phenotype that will actually develop, and in some cases this has been demonstrated. Genetic alterations in the VHL gene in type-1 families mostly constitute deletion, nonsense or frame-shift mutations, whereas 90% of VHL mutations

found in type-2 families are mis-sense mutations [25, 76, 77]. A few mis-sense mutations have been described that specifically lead to VHL type 2A [25].

The VHL gene encodes two different protein isoforms. Both isoforms suppress RCC growth in vivo and are referred to as pVHL [78, 79]. The pVHL functions as a component of an ubiquitin ligase complex (VBC complex), and its best understood function relates to its ability to target and destruct specific proteins [80]. The pVHL forms stable complexes that contain other

Table 3. Main protein targets of the VHL

Target protein	Function
Hypoxia inducible factors 1 and 2	Protein transcription
Vascular endothelial growth factor	Angiogenesis
Glucose transporter protein-1	Glucose transport
Platelet-derived growth factor- β	Angiogenesis, mitogenesis
Transforming growth factors α and β	Epithelial proliferation, mitogenesis
Intracellular fibronectin	Epithelial proliferation
Erythropoietin	Erythropoiesis
Carbonic anhydrases 9 and 12	Extracellular pH control
Chemokine receptor	Metastasis

proteins entitled elongin B, elongin C, Cul2, and Rbx1 [81, 82]. These complexes direct the covalent attachment of polyubiquitin tails to specific proteins, serving as a signal for such proteins to be degraded by the proteasome.

Several pVHL targets have been identified, including the members of the hypoxia-inducible factor- α (HIF- α) family (HIF-1 α , HIF-2 α , and HIF-3 α). The HIF-1 α and HIF-2 α , when bound to a HIF- β member, form a sequence-specific, DNA-binding transcription factor called HIF. Ordinarily, the HIF- α family members are highly unstable except under low-oxygen conditions. In the presence of oxygen, these proteins become hydroxylated on conserved prolyl residues in a reaction catalyzed by members of the EGLN family [83–86]. pVHL recognizes the hydroxylated HIF- α species and orchestrates their destruction through ubiquitination [87–89].

Since the 1990s, evidence has continued to accumulate demonstrating that hypoxia is a common consequence of the rapid growth of renal cancer (fig. 2) [90, 91]. Molecular studies provide evidence that hypoxia is an important regulator of a network of gene expression with the ability to stimulate and inhibit individual genes and to effect their expression on both posttranscriptional and post-translational levels [90]. Among these genes are those that control glucose uptake and metabolism (such as the Glut1 glucose transporter and various glycolytic enzymes), extracellular pH (such as carbonic anhydrase IX), angiogenesis (such as vascular endothelial growth factor, VEGF), erythropoiesis (such as erythropoietin), and mitogenesis (such as TGF- α and PDGF-B). Signaling occurs through various cell surface receptors, including EGFR, HER2, VEGFR and type-I insulin-like growth factor receptor. During normoxia, the VHL complex targets and degrades HIF. In cells that lack pVHL, or when oxygen is limiting, the complex cannot degrade HIF.

HIF-1 α and 2 α are upregulated through the phosphatidylinositol 4,5-bisphosphate-AKT-mammalian target of rapamycin pathway (PI3K-AKT-mTOR) and Ras/Raf/MEK/mitogen-activated protein kinase (MAPK) pathway and they form the transcription factor HIF. HIF is free to accumulate intracellularly and activates the transcription of a cadre of genes that encode proteins essential for cancer cell functions under hypoxic conditions (table 3) [92, 93]. These considerations explain the frequent overproduction of HIF, as well as its downstream targets, in RCC [80].

The pVHL binds to other cellular proteins, some of which may also be polyubiquitinated or in some other way modulated by pVHL. These proteins include the atypical protein kinase C family members [94], Sp1 [95], heteronuclear ribonucleoprotein hnRNP A2 [96], specific RNA Pol II subunits (Rpb1 and hsRBP7) [97, 98], Jade-1 [99], Vdu1/2 [100], fibronectin [101], proteins associated with microtubules [102], nuclear factor κ B [103, 104], and metalloproteinases [105].

Recently, it has been demonstrated that VHL inactivation in RCC cells disrupts intercellular junctions and cell shape through HIF-independent events, supporting the concept that VHL has additional functions beside its role in the regulation of HIF [106]. Although our knowledge of the functions of pVHL is still incomplete, studies conducted thus far highlight its role as an inhibitor of HIF, and dysregulation of HIF is likely to contribute to the development of RCC [107].

Hereditary PRCC

Patients with HPRC are at risk for development of bilateral, multi-focal type-1 papillary renal carcinoma [45, 108]. Genetic linkage analysis in HPRC families identi-

fied the proto-oncogene, c-MET (located at 7q31.1–34) to be responsible for the hereditary form of type-1 papillary renal carcinoma [109]. MET belongs to the family of tyrosine kinases, which transmit signals from the cellular surface to the nucleus [110].

Most HPRC-associated MET mutations are mis-sense mutations located within the tyrosine kinase domain. MET mutations do not require concomitant loss of the remaining MET allele, as is true for the VHL gene. Instead, chromosome 7 duplications leading to trisomy 7 have been found to be associated with gain-of-function MET mutations in a majority of HPRC tumors. Genotyping analyses have demonstrated that chromosome 7 carrying the mutant MET allele is duplicated in these papillary renal tumors [111, 112].

MET acts as a transmembrane receptor for the hepatocyte growth factor. Binding of this growth factor induces cellular mitogenesis, morphogenesis and cellular migration, through a growth-stimulating signaling cascade via RAS. It was suggested that mutations in the cytoplasmic kinase domain of the MET gene might lead to constitutive activation without ligand binding and, thus, to continued activation of the signaling cascade leading to out-of-control cellular proliferation. Transfection studies in combination with biological assays showed that carcinogenesis is related to enzymatic activation of MET [113].

Hereditary Leiomyomatosis RCC

HLRCC is an inherited cancer syndrome in which patients are at risk for the development of cutaneous and uterine leiomyoma and type-2 papillary RCC [114]. Genome linkage analysis linked the syndrome to 1q42–44 [115] and identified mutations in the gene for fumarate hydratase (FH) as the causative abnormality [116, 117]. These mutations were found to reduce the activity of FH, an enzyme in the tri-carboxylic acid (Krebs) cycle that is involved in energy production and amino acid metabolism. Reduced enzyme activity and associated LOH at 1q42–44 in uterine leiomyomas and renal tumors support the function of FH as a tumor-suppressor gene.

Types of germline FH mutations in HLRCC include protein-truncating mutations, large and in-frame deletions, and mis-sense mutations [115]. Mis-sense FH mutations predisposing to renal cancer have no unusual features, and identical mutations were found in families without renal cancer, suggesting a role for genetic or en-

vironmental factors in renal cancer development in hereditary leiomyomatosis [117].

Activation of the hypoxia/angiogenesis pathway may contribute to carcinogenesis in HLRCC. Studies have demonstrated increased expression of transcripts from the hypoxia-responsive genes VEGF and BNIP3. It is possible that failure of the Krebs cycle in HLRCC tumors causes inappropriate signaling of a hypoxic state, leading to angiogenesis and perhaps directly to clonal expansion and tumor growth through a cell-autonomous effect [118]. However, it remains to be elucidated how abnormalities in FH protein expression play a role in HLRCC-related carcinogenesis.

BHD Syndrome: Chromophobe and Oncocytic Renal Carcinoma and Oncocytoma

The BHD gene (also known as FLCN) has been mapped at 17p12–q11.2 [66]. Oncocytomas and chromophobe RCC are the most frequent renal tumors in BHD syndrome, but hybrid-oncocytic renal carcinoma, papillary type 1, and clear-cell RCC have also been reported [119–121]. Cytogenetic studies on the clear-cell RCCs have revealed that they are identical to sporadic conventional RCCs, with frequent 3p loss and concomitant VHL inactivation and 5q gain [122]. Studies on hybrid oncocytic tumors have shown few or no consistently found cytogenetic abnormalities [68].

BHD gene alteration is not specific for particular histological types of RCC, unlike abnormalities in VHL (clear-cell) or c-met (papillary) [120]. There is evidence that it acts as a tumor-suppressor gene [122, 123]. The majority of the mutations detected were predicted to prematurely terminate the BHD protein and to result in loss of its function [120]. Loss of the wild-type BHD allele or somatic frame-shift second-hit mutations were confirmed in renal tumors of relatives with BHD, resulting in bi-allelic inactivation of BHD [120].

Mutation analysis of the BHD gene has revealed frame-shift or termination mutations in the majority of BHD families [66]. There is evidence for possible genotype-phenotype associations. A mutational hotspot, a C insertion/deletion in exon 11, is frequently mutated in BHD families. Renal tumors developed with a higher frequency in C-insertion carriers compared with C-deletion carriers, and more frequently in individuals who inherited a putative splice-site mutation in intron 9. The residual mutant protein produced in patients with the C-insertion mutation might have a dominant-negative effect and in-

terfere with the wild-type BHD protein function, leading to the development of renal tumors. Alternatively, the residual mutant protein encoded by the C-insertion mutation might predispose kidney cells to form a renal tumor [120].

The BHD gene product, folliculin, is highly conserved, and reduced BHD expression was demonstrated in renal tumors from patients with BHD, regardless of the histological type [125]. Nevertheless, the protein does not belong to any known protein family, and its exact function remains to be elucidated [68].

Other Hereditary Renal Cancers

Germline Constitutional Chromosome 3 Translocations

Clear-cell RCC segregation was found in families that show constitutional balanced translocations involving chromosome 3 [126]. The abnormalities found included: t(3;8)(p14;q24) [127], t(3;6)(p13;q25.1) [128], t(2;3)(q35;q21) [129], t(3;4)(p13;p16) [130], t(2;3)(q33;q21) [131], t(3;6)(q12;q15) [132] and t(1;3)(q32;q13.3) [133].

Molecular studies in these families revealed that some of them carried different VHL mutations in the allele derived from the remaining 'normal' chromosome 3. The concurrent observations of loss of 3p derivative chromosomes and somatic VHL mutations led to the proposition of a three-step model for carcinogenesis [4, 25, 134, 135]. The first hit would be the occurrence of a germline chromosome 3 translocation, while non-disjunctional loss of the derivative chromosome that carries the 3p segment would represent the second step. Finally, the third step would involve a somatic mutation in the remaining 3p allele of an RCC-related tumor-suppressor gene such as VHL. However, since some of the analyzed familial tumors did not carry VHL mutations, it seems likely that other tumor-suppressor genes located in the 3p arm could also be involved in the development of conventional RCC.

Familial Clear-Cell RCC with No Known Genetic Abnormality

Several families have been reported with a cluster of clear-cell RCCs in which the renal tumors were unrelated to germline VHL mutations, chromosome 3 translocations or MET mutations [10, 11]. These tumors may represent an increased predisposition to develop a first hit in unknown genes within renal epithelial cells that leads to carcinogenesis.

Renal Medullary Cancers in Sickle Cell Trait/Disease

Around 90 cases of advanced renal medullary cancer have been reported, mostly in young, male Africans with sickle cell trait, some with sickle cell disease or other hemoglobinopathies [136].

Novel Molecular Therapeutics

Molecular targeting against RCC has already been studied in several clinical trials (table 4). The VHL pathway can be targeted by blocking the transcription of HIF through inhibition of the mTOR pathway with rapamycin and analogues [93, 137], inhibition of the ubiquitin/proteasome system with bortezomib [138] and inhibition of heat shock protein 90 with geldanamycin and 17-(allylamino)-17-demethoxygeldanamycin [139]. A number of other agents downregulate HIF, including HDAC inhibitors [140], topoisomerase I inhibitors [141], thioredoxin-1 inhibitors [142], and microtubule disruptors [143].

The downstream targets of HIF, such as VEGFR, EGFR and PDGFR, can also be manipulated in several ways. BAY 43-9006 is a potent inhibitor of the cellular serine/threonine kinase receptor pathway, and is active against VEGFR and PDGFR, leading to anti-angiogenesis, the inhibition of tumor cell proliferation [144]. Bevacizumab, a neutralizing antibody against VEGF, has been shown to delay progression of metastatic RCC [145].

Cetuximab and gefitinib, which target EGFR have showed no activity against RCC [146, 147]. Combination agents such as ZD6474 that can inhibit at least two arms of the downstream HIF pathway (i.e. VEGFR and EGFR) are currently under clinical evaluation [148]. SU-011248, an orally bioavailable indolinone acting as a signal transduction inhibitor of VEGFR, PDGFR and the c-kit tyrosine kinase, showed encouraging results and is under evaluation in a phase-III trial [149]. Thalidomide is also a potent in vivo angiogenesis inhibitor [150]. Due to its critical role in RCC pathobiology, carbonic anhydrase IX has been exploited as a potential target for immunotherapy [151, 152].

Studies upon the activating point mutations in c-MET found in type-1 papillary RCC suggest at least three strategies for therapeutic development: (a) block kinase activation with small molecule inhibitors of ATP binding; (b) block HGF-MET interaction, and (c) block interactions between activated c-MET and downstream intracellular signaling molecules [153].

Table 4. Novel molecular therapeutics for RCC

Category	Mode of action
<i>Inhibition of the hypoxia-inducible pathway</i>	
Rapamycin	Inhibits PI3K-AKT-mTOR signal transduction pathway
Rapamycin analogues CCI-779 RAD001	Inhibits PI3K-AKT-mTOR signal transduction pathway
Raf kinase inhibitors (BAY 43-9006)	Targets Raf kinase + VEGFR-2
Proteasome inhibitors (bortezomib, PS-341)	Inhibits 26S proteasome catalytic activity, thus, preventing proteolysis
<i>Epidermal growth factor receptor (EGFR) inhibitors</i>	
Gefitinib, ZD1839	Inhibits EGFR tyrosine kinase
Erlotinib, OSI 774	Inhibits EGFR tyrosine kinase
<i>Monoclonal antibody therapy</i>	
WX-250	Antibody that targets VEGF
Bevacizumab	Neutralizing antibody that targets VEGF
Panitumumab, ABX-EGF	Antibody that targets EGF receptor (EGFR)
MDX-010	Antibody that targets CTLA-4 to block lymphocyte activity
Cetuximab (C225), IMC-C225	Antibody that targets EGFR
<i>Anti-angiogenesis agents</i>	
Thalidomide	Immunomodulatory agent with multiple actions, including VEGF + basic fibroblast growth factor inhibition
Thalidomide analogues (CC5013)	Immunomodulatory agent with multiple actions, including VEGF + basic fibroblast growth factor inhibition
Endothelin-1 receptor antagonists (atrasentan, ABT-627)	Selective endothelin-1 receptor antagonist
Indolinone (SU-011248)	Inhibits VEGFR, PDGFR + FLT3 signal transduction, + c-kit tyrosine kinase
VEGFR + EGFR inhibitors (ZD 6474)	Targets VEGFR 1,V EGFR
VEGFR inhibitors (PTK 787)	Selectively targets VEGFR 1, VEGFR-2 + VEGFR-3 tyrosine kinases

Epilogue

Numerous molecular studies upon RCCs have been performed, making RCC one of the best genetically understood cancers. With the availability of advanced molecular tools such as genomics, transcriptomics and proteomics the molecular pathogenesis of RCC has been elu-

cided. The understanding of RCC carcinogenesis might lead to a novel tumor classification and to better treatment outcomes with the aid of novel anticancer agents. The role of surgery before or after administration of novel molecular agents in metastatic disease, and the role of these agents in the neo-adjuvant or adjuvant setting in localized disease will need further evaluation.

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