

The genetic basis of renal epithelial tumors: advances in research and its impact on prognosis and therapy

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The genetics of renal cell carcinoma continues to elucidate the pathways of kidney tumorigenesis. The relationship between the *VHL* gene and clear cell carcinoma, *MET* and papillary carcinoma, and the families of genes that they regulate, continues to be unraveled. New hereditary kidney cancer syndromes, like familial oncocytoma and the Birt-Hogg-Dubé syndrome, have been identified and the search for the genes that cause them is under way. Researching the genetics of these disorders is essential for an understanding of sporadic kidney cancer genetics. This chapter will review the current knowledge of the hereditary kidney cancer syndromes, the genes that cause them, new advances in genetic research and techniques, and how this information impacts upon diagnostic, prognostic, and therapeutic methods of the future. *Curr Opin Urol* 11:463-469. © 2001 Lippincott Williams & Wilkins.

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Abbreviations

BHD	Birt-Hogg-Dubé (syndrome)
FCRC	familial clear cell renal cell carcinoma
FRO	familial renal oncocytoma
HCRCC	hereditary clear cell renal cell carcinoma
HGF	hepatocyte growth factor
HPRC	hereditary papillary renal cell carcinoma
LQH	loss of heterozygosity
RCC	renal cell carcinoma
TSG	tumor suppressor gene
VHL	von Hippel-Lindau (disease)

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Introduction

With the advent of the Human Genome Project, molecular oncology will move into a new phase in which the genetic fingerprint of a patient's tumor may be exploited for its full diagnostic, prognostic and therapeutic potential. For renal cell carcinoma, which has no known cure when metastatic, an understanding of genetics is particularly relevant. This review focuses on the genetics of renal cell carcinoma, how the different epithelial kidney cancers are classified according to known genetic pathways, and how emerging technologies may facilitate laboratory to bedside genetic therapies. Discussed are the hereditary kidney cancer syndromes, von Hippel-Lindau (VHL) disease, hereditary papillary renal cell carcinoma, and Birt-Hogg-Dubé (BHD) syndrome, and how an understanding of these may impact on sporadic carcinomas and influence their treatment.

Renal cell carcinomas: genotypes

A great leap in our understanding of sporadic renal cell carcinomas (RCCs) has come from researching hereditary RCCs, including those seen in VHL disease, hereditary papillary renal cell carcinoma (HPRC), BHD syndrome, and hereditary clear cell renal cell carcinoma (HCRCC). This work has led to the identification of at least two genes highly important in sporadic kidney cancer. Furthermore, the genetics of RCC illustrate some of the bases of how an imbalance of positive and negative genetic signals favors unregulated cellular growth and carcinogenesis.

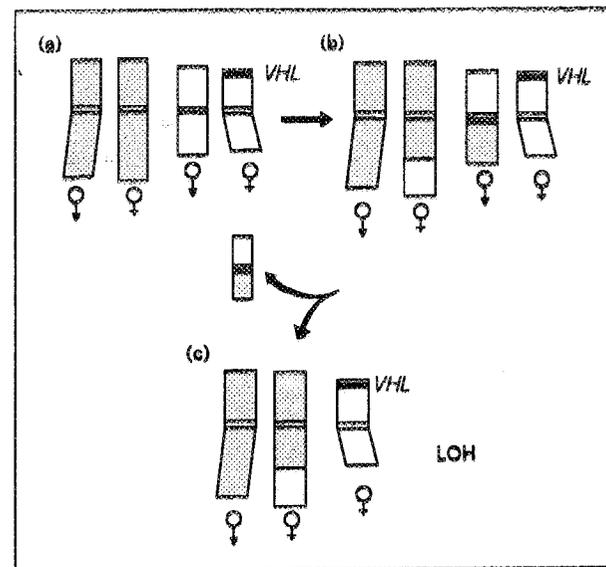
Clear cell carcinoma: inactivation of *VHL*

The classic model of tumor suppressor genes suggests that loss or inactivation of both maternal and paternal copies of the tumor suppressor gene (TSG) are required in order to establish a tumor phenotype [1,2]. In the case of hereditary cancers, patients inherit a defective TSG in every cell (e.g. germline loss) but do not develop tumors until the other allele becomes mutated or deleted (e.g. somatic loss). The two-hit hypothesis appeared to be particularly relevant to the most common hereditary kidney cancer, VHL disease. In unrelated families with clear cell carcinomas, constitutional translocation of the short arms (*petite*, p) of chromosome 3 was identified involving the same 3p breakpoint [3-5]. Using DNA markers, the region of interest was limited to 3p25, which led to the identification of a new gene common to

kidney cancer patients: the *VHL* gene [6–8]. *VHL* is involved in a transcription-regulatory complex which includes elongin B/C and Cul-2 [9,10]. Multiple roles for *VHL* are still being defined and include regulation of hypoxia-sensitive genes, angiogenesis, apoptosis, glucose metabolism, and growth factor metabolism [11,12–15,16,17,18].

As the molecular profiles of *VHL* and its pathway emerge, an understanding of the basis of *VHL* inheritance helps shed light on how sporadic cancers form. Two copies of *VHL*, like all genes, appear as distinct bands on a DNA gel reflecting the heterozygous nature of most human alleles. Patients with *VHL* disease, by definition, have inherited a mutated *VHL* gene or a *VHL* gene that is partially or completely deleted [19,20]. Every cell in a *VHL* patient's body is thus haplo-insufficient for *VHL*. In contrast, DNA from *VHL* tumors has been shown to retain the abnormal, inherited copy and to have lost the normal, unaffected *VHL* copy [21,22–25]. Thus, the heterozygous two-banded DNA pattern on a gel is lost in *VHL* tumors. Loss of heterozygosity (LOH) is thought to be the sentinel event, or 'second hit', which commits the normal but *VHL* haplo-insufficient cell to carcinogenesis (Fig. 1) [26]. In sporadic kidney cancer, LOH of *VHL* occurs in the majority of specimens, although the exact pathway from wild-type (i.e. normal/normal) to LOH may be distinct from that seen in hereditary cases [27–29,30]. In sporadic cases, it is believed that the 'first hit' or inactivation of *VHL* occurs after an acquired mutation of either *VHL* allele. The haplo-insufficient or hemizygous state continues until the second allele is lost through an unbalanced chromosomal translocation, methylation or, far less likely, a second *VHL* mutation or deletion [19,21,31,32] (for color figures, see <http://www.riedlab.nci.nih.gov/Publications>). In the *VHL* kidney, LOH of the *VHL* gene can be demonstrated in tiny, oligocellular cysts but not in histologically 'normal' material [22]. There is evidence that in a few sporadic tumors, pathways other than LOH of *VHL* may play a role, and a small percentage of lesions in the *VHL* kidney may develop chromosomal abnormalities prior to developing LOH [30,33–35]. Ultimately it may not depend on the exact nature or order of the first or second hits in familial or sporadic kidney cancer, but the cumulative 'hits' which occur thereafter. For this reason, early kidney cancers from inherited and sporadic cases merit study to identify any other changes in the young cell that occur after *VHL* gene inactivation [36]. Described changes in sporadic renal cell carcinoma include increased activity of growth promoting genes (i.e. oncogenes like *FHIT*, *c-fos*, *AP-1* and *c-myc*); loss of cell cycle arrest and cell death genes (e.g. *TRAIL*, *bcl-2*); genes promoting angiogenesis (e.g. *VEGF*, *PDGF*) and numerous genes involved in the regulation of protein,

Figure 1. 'Two hit' model of tumorigenesis in von Hippel-Lindau disease



(a) 'First hit': patients with von Hippel-Lindau (*VHL*) disease inherit a mutated *VHL* allele (thick black bar on light gray chromosome 3) and are thus said to be haplo-insufficient for *VHL*. (b) In a cell of an affected organ (e.g. kidney), a cytogenetic event(s) occur(s) involving the normal allele, here shown as a balanced translocation involving the q arm of chromosome 2 (dark gray chromosome) and the q arm of chromosome 3 [breakpoint indicated by light arrowheads in (a)]. (c) 'Second hit': cells that lose the derived chromosome carrying the normal 3p allele retain only the abnormal *VHL* allele. Loss of one parental allele defines loss of heterozygosity (LOH) and is the obligatory event for tumorigenesis. What next steps follow are unknown but hold the key to understanding cancer formation in all tissues.

fatty acids, and carbohydrates; oxygen radical formation; genes promoting invasion or metastasis; and genes which appear to allow the cancer cell to escape identification by the immune system [35,37–42,43,44–47]. Furthermore, it may not be the exact number of genes which are abnormally on or off in a cancer cell but the sequence in which these changes occurred that determines a tumor's overall clinical behavior. Given the formidable genetic biology that a cancer cell develops before it becomes clinically relevant, therapies may need to include more than replacement of missing function of *VHL*. In this aim, the study of other hereditary kidney cancer syndromes may yield complementary insights applicable to all clear cell carcinomas.

Papillary renal cell carcinoma: *MET* oncogene activation

In contrast to *VHL* disease, in which the 'two-hit' hypothesis describes the requisite loss of two copies of a TSG, papillary RCC may follow a different pathway [48,49]. As in *VHL* disease, HPRC tumors are often bilateral and present with tumors ranging from low to

higher stage within the same renal unit. Unlike in clear cell RCCs, no constitutional chromosomal translocations or germline mutations of the *VHL* gene were seen in HPRC family members. Karyotypic analysis of HPRC tumors revealed that of the few karyotypic abnormalities, gains of chromosome 7 were most common [50]. A candidate gene region at 7q31 was soon identified which contains the oncogene *MET* and which appears mutated in HPRC members [51]. *MET* codes for the cellular receptor for hepatocyte growth factor (HGF). Mutations of *MET* in HPRC families give rise to mutant HGF receptors which are unable to 'turn off' from the activated state after HGF binding. HGF is expressed in many tissues, appears to be a cellular mitogen, induces morphologic changes in responsive cells, and increases cellular migration [52,53].

Such unchecked positive growth signals could potentially drive HGF-responsive cells into unregulated cell cycling, morphogenesis, and eventual tumorigenesis. In this way, *MET* may function as a positive effector or oncogene for tumor growth, in contrast to the suppressive effects of the *VHL* gene in clear cell carcinoma. *MET* mutations need not, therefore, require sequential losses of *MET* DNA, as with the *VHL* gene, but rather only one copy of a defective gene coding for mutant receptor. Study of sporadic papillary tumors revealed that most tumors did not have mutations in *MET* but two- to three-fold gains of chromosome 7 or the 7q31 region [54]. Thus, in addition to mutant *MET* receptors being tumorigenic, gains of DNA coding for normal *MET* receptors may be tumorigenic when the result is increased cellular responsiveness to HGF. This may explain the relative paucity of *MET* mutations in sporadic papillary tumors, reflecting that gains of normal copies may be sufficient for the cancer causing steps [55] (for color figure, see <http://www.riedlab.nci.nih.gov/Publications>). The majority of HPRC families pass on *MET* genes with activating mutations. A small number of families with papillary renal neoplasia, however, have no detectable *MET* gene changes. Tumors from non-*MET* mutant papillary families often appear histologically different from those with *MET* mutations, and may be clinically more aggressive [56]. The search for the other gene or genes downstream from *MET* is therefore important for both hereditary and sporadic forms. Loss of 3p or *VHL* does not appear in typical papillary lesions, however chromosomal losses of Y, 6q, 9p, 1p, and Xp have been demonstrated [57,58]. Loss of Xp appears to be associated with a particularly fulminant clinical course. Chromosomal gains are typically seen with chromosomes 7 and 17, and more specifically, the 17p region, especially those which appear in hemodialysis patients with acquired cystic kidney disease [59,60]. Interestingly, the role of p53, the major cell cycle regulator found at 17p13.1, has not yet been defined

conclusively in sporadic papillary or clear RCC [28,61,62]. Some papillary tumors have increased expression of the angiogen, vascular endothelial growth factor, as well as oncogenes like *c-myc* and *c-fos*, all three of which are upregulated in clear cell carcinomas [63,64]. Such data illustrate that clear cell and papillary cancers, associated with loss of the *VHL* gene and activation of the *MET* gene, respectively, appear to arise from different genetic causes but may converge on similar genes along the pathway to unique histologies.

Oncocytoma: multiple tumor suppressor gene candidates

Renal oncocytomas, benign tumors arising from intercalated cells of the cortical collecting duct, may develop in patients with familial renal oncocytoma (FRO) [65]. FRO is suspected in patients who have bilateral or multifocal oncocytomas or among family members who have histologically verified oncocytomas. Patients with FRO, who have small, asymptomatic masses, have been managed with observation, and undergo attempts at nephron-sparing surgery only when intervention is necessary. Renal oncocytomas often grow slowly, and fortunately patients with FRO uncommonly require surgery. Therefore, compared to clear cell or papillary carcinomas, less is known regarding oncocytoma cytogenetics. Three subgroups of oncocytomas, however, have been described: those with losses of chromosomes 1 or Y, those with translocations involving 11q13, and those with more heterogeneous abnormalities, including monosomies, trisomies, and losses at 17p, 17q, 10q, and rarely 3p [56]. These and other chromosomal regions such as 5q translocations and 14q losses figure prominently in sporadic clear cell carcinoma as well, especially 5q, which is amplified in over 80% of clear cell cases [66]. Loss of 1p36, which is seen in 20-30% of sporadic clear cell carcinoma and >50% of most cases of Wilms' tumor, may cause loss of growth-regulating genes [67].

Chromophobe renal cell carcinoma: loss of multiple chromosomes and a near haploid genome

Sporadic chromophobe RCCs are malignant tumors arising from either cortical collecting ducts or their intercalated cells, which can have a prolonged or fulminant course with metastatic potential. Metastases from chromophobe carcinomas, though rare, tend to be found preferentially in the liver, unlike clear cell tumors that metastasize to regional lymph nodes, lung, bone, and brain [68,69]. Chromosomal and genomic DNA analyses have shown that chromophobe tumors are unique in having a near haploid genome, with frequent losses of chromosomes 1, 2, 6, 10, 13, 17, and 21 [70]. These multiple losses have made it difficult to define the essential genetic 'hit' for this disease. A recently described inherited disorder, however, the BHD syndrome, may shed light on this particular neoplasm.

The BHD syndrome was originally described in families who inherited a tendency to develop multiple cutaneous fibrofolliculomas and trichodiscomas, benign lesions distributed about the face, neck, back, and chest [71]. Recent studies of affected families confirm the autosomal dominant nature of the disorder, and suggest that the disease also predisposes to lung cysts, spontaneous pneumothorax, and multifocal or bilateral renal cancers. The renal lesions removed from patients with BHD syndrome are primarily chromophobe RCCs, although clear cell tumors and hybrid chromophobe/oncocytic tumors are also commonly found [C. Pavlovich (Johns Hopkins, USA), personal communication]. Thus, chromophobe carcinoma, like tumors in VHL, HPRC, and FRO families, may arise from a gene that may be mutated either sporadically or in inherited fashion. Positional cloning efforts using multipoint linkage analysis of BHD families, and the study of BHD tumor cytogenetics, may lead to the identification of a new kidney cancer gene.

Hereditary and familial clear cell renal cell carcinoma

Cases of clear cell renal cell carcinoma appear as clusters in some families who do not have mutations in *VHL* or *MET* [72,73]. Constitutional, balanced translocations involving chromosome 3 have been identified in some of these families (Hereditary or HCRCC) [3,72,74], while normal karyotypes are found in other families (Familial or FCRC). Chromosomal analysis of families with predispositions to clear cell RCC reveals little to differentiate them from sporadic renal tumors, but germline balanced translocations lead to loss of derivative chromosome 3 in the renal tumors [21]. These syndromes may thus predispose to RCC by increasing the chances of a tumorigenic mutation in a renal epithelial cell. The study of family members of HCRCC and FCRC families and their tumors may lead to the identification of genes which are important in VHL-related and unrelated sporadic clear cell carcinomas.

Renal cell carcinoma research: new techniques of discovery

Advances in DNA and chromosomal analysis, imaging, and microchip technology, and publication of the DNA sequence of the entire human genome create an unparalleled opportunity for discovery. Cytogenetic research of the past relied upon classic G-banding of chromosomes, a notoriously challenging endeavor with solid tumor karyotypes. Spectral karyotyping, described in 1996 and only now applied to RCCs, allows the simultaneous visualization of all chromosomes in unique colors [30,75] (for color figure, see <http://www.riedlab.nci.nih.gov/Publications>). Spectral karyotyping is invaluable in identifying chromosomal breakpoints that were too complex or subtle for traditional techniques and refine the search for new genes [76]. Comparative

genomic hybridization allows one to assess which chromosomal regions are gained or lost in tumors [30,66,77]. A combination of spectral karyotyping and comparative genomic hybridization data, in an Internet-accessible database, has tremendous diagnostic and prognostic potential (see international interactive database at <http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi>). Microarrays of cDNAs allow the simultaneous assessment of thousands of genes, or in the case of tissue microarrays, hundreds of tumor samples [78,79]. Now researchers have the tools to understand the cancer cell in real time: the simultaneous interaction and clustering of gene expression patterns to provide a cancer cell with a growth advantage.

Renal cell carcinoma therapy: bench to bedside

There is no medical cure for all patients with RCC and only patients with organ-confined disease who undergo nephrectomy will uniformly experience long-term survival. Of all therapies for metastatic disease, only IL-2 based immunotherapy is currently approved by the US Food and Drug Administration for treatment of this disease. Therefore, better therapies are required for almost all patients, including even those who are surgical candidates. Molecular targeting based therapy has the attraction of targeting the cancer for what causes it: a dysregulation of genes important to kidney homeostasis. It is too early to tell whether or not replacement of the function of missing *VHL* or a molecular blocking of *MET* action will be sufficient to arrest tumor growth. Although causal for tumor formation, *VHL* and *MET* are certainly among many genes that have become dysregulated by the time tumors reach clinical significance. An alternative approach would be the design of therapies to make RCCs more susceptible to conventional therapies such as radiation or chemotherapy, against which RCCs are notoriously refractory. Preliminary molecular targeting studies suggest that such strategies may enhance immune-cell mediated tumor killing and sensitivity to alkylating agents [80-85]. Promising recent reports of immune-based therapy have relied on cell-based rather than molecular therapies. As recently demonstrated by two groups, approximately 40% response rates in metastatic RCC have been shown using non-myeloablative stem cell transplantation or allogeneic dendritic-cell tumor cell fusion vaccines. These strategies may in the future be further improved with additional genetic manipulation [86,87].

Conclusion

Prior to the identification of the *VHL* tumor suppressor gene, little was known about the genetics of sporadic clear cell RCC. We know now that a majority of clear cell carcinomas develop in association with loss of function of both copies of *VHL*. Correspondingly, we know now that

some papillary RCCs arise through unchecked growth stimulation via mutated or extra copies of the MET hepatocyte growth factor receptor. Soon, a third gene inherited by patients with chromophobe-type carcinomas may be described. None of these discoveries would have been possible without the detailed study and care of patients with inherited kidney cancer syndromes in which these genes figure so prominently. With the identification of such hereditary genes, the complex biology of sporadic kidney cancers will be slowly unraveled. The next frontier of kidney cancer genetics will be the isolation of new tumor suppressor and oncogenes and their modifiers, the definition of how they interact in real time, and the sequences in which their dysregulation defines tumor phenotype and clinical course. In this way, the true diagnostic, prognostic, and therapeutic potential of kidney cancer genetics can be realized.

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