Current understanding of the molecular mechanisms of kidney cancer: a primer for urologists

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Abstract

Renal cell carcinoma (RCC), the fifth leading malignant condition for men and tenth for women, accounts for 3% of all malignancies in Canada. It is a heterogeneous epithelial malignancy with different subtypes and varied tumour biology. Although most cases of RCC are sporadic, up to 4% of patients have an inherited predisposition for the disease. In this article, we review the current molecular genetics of the different subtypes in hereditary and sporadic RCC. Significant developments in understanding the underlying genetic basis of RCC over the last 2 decades are attributed to intensive research about rare inherited renal cancer syndromes and the identification of the genes responsible for them. Many of these genes are also found in sporadic RCC. Understanding the molecular mechanisms involved in the pathogenesis of RCC has aided the development of molecular-targeted drugs for this disease.

Renal cell carcinoma (RCC), the fifth leading malignant condition in men and the tenth in women, accounts for 3% of all malignancies in Canada.[1] Though the mortality rate has been stable, the incidence of renal cancer has increased steadily for both sexes.[1] In 2006, 4600 new cases were detected and resulted in 1500 deaths. The male to female ratio is roughly 2:1. Age-specific incidence shows a peak in early childhood, then follows the more usual pattern of a steep rise through adulthood.[1]

According to Heidelberg classification,[2] malignant renal epithelial neoplasms are subclassified into common or conventional RCC, comprising 75% of renal cell neoplasms; papillary RCC (10%); chromophobe RCC (5%); collecting-duct carcinoma with medullary carcinoma of the kidney (1%); and RCC, unclassified (3%–5%).

Most cases of RCC are sporadic; up to 4% of patients have an inherited predisposition for this disease, including families with von Hippel-Lindau (VHL) disease, hereditary papillary renal cancer (HPRC), hereditary leiomyomatosis and renal cancer (HLRCC), and Birt-Hogg-Dubé (BHD) syndrome. The study of renal cancer genes and their molecular mechanisms is of utmost importance because it may help identify targets for therapy that will improve survival.

Hereditary kidney cancer

von Hippel-Lindau disease

The discovery of VHL disease occurred in 1894 when Collins reported a case of a bilateral vascular tumour in the retinas of 2 siblings.[3] Since then, the constellation of lesions associated with this hereditary disease include hemangioblastoma of the central nervous system, visceral cysts or tumours, pheochromocytoma, endolymphatic sac tumours of the inner ear, epididymal and broad-ligament cystadenomas, and clear-cell RCC.[4] VHL disease is a rare autosomal-dominant syndrome affecting approximately 1 in 35 000 people.[5]

The *VHL* gene, a tumour-suppressor gene,[6,7] was mapped by linkage analysis to chromosome 3p25[8] and was isolated in 1993 with a positional cloning strategy.[9] Its product, VHL protein (pVHL), consists of an α and β domain, and resides primarily in the cytoplasm, but can shuttle between the cytoplasm and the nucleus,[10–13] This activity is important to its function in regulating transcription and production of growth factors.[14]

Through its α domain, pVHL forms a stable complex with Elongin subunits B and C,[15–17] Cullin 2,[18,19] and RING-box protein Rbx1[20] to form a ubiquitin E3 protein-ligase complex. pVHL serves as the substrate receptor of the complex that directly binds to specific proteins, such as hypoxia-inducible factor (HIF), and subsequently targets them for degradation through the ubiquitin proteolytic pathway.[21]

Hypoxia-inducible factor-1 (HIF-1) is a transcriptional activator that plays a crucial role in mediating cellular response to oxygen. HIF-1 is made up of a hypoxia-inducible subunit HIF-1α and a constitutively expressed subunit HIF-1β.[22] Under adequate oxygena-

tion, HIF-1α is hydroxylated by an oxygen-dependent prolyl hydroxylase in its 2 proline residues (Pro^{402},Pro^{564}) located within the oxygen-dependent degradation domain in the cytoplasm,[23,24] which then permits binding with the β domain of pVHL. This promotes the ubiquitination and destruction of HIF-1α. Also, hydroxylation by asparagine hydroxylase in the nucleus regulates its interaction with coactivator p300 and reduces the transcriptional activity of HIF-1.[25]

Patients with VHL disease were found to carry a germline mutated *VHL* allele and a wild-type *VHL* allele. Inactivation or loss of the wild-type *VHL* allele results in failure to bind with HIF-1α. Consequently, HIF-1α accumulates under normoxia, translocates from cytoplasm to nucleus and subsequently heterodimerizes with HIF-1β to enable binding to an HIF-responsive element[26] and induce transcription of target genes leading to the production of GLUT-1 glucose transporter,[27] platelet-derived growth factor β, carbonic anhydrase genes *CA IX* and CA *XII*,[28,29] vascular endothelial growth factor (VEGF),[30,31] and transforming growth factor α,[32] thereby contributing to renal carcinogenesis.

pVHL has been involved in numerous cellular processes such as regulation of the extracellular matrix (ECM); cytoskeletal stability; and cell-cycle control and differentiation, other than regulation of HIF. Studies[33,34] have shown that dysregulation of HIF does not completely explain the tumorigenesis in *VHL*-deleted cells,[35,36] which suggests that pVHL may have HIF-independent tumour-suppressor functions.

A recent study[37] has shown that pVHL in RCC cell lines is necessary for the normal organization of adherence and tight intercellular junctions, maintenance of cell polarity and control of paracellular permeability. Loss of *VHL* function leads to abnormalities in the deposition of extracellular fibronectin, a glycoprotein that interacts with integrins to bridge cells to the structural proteins of the ECM.[38] A study[39] reported that pVHL is required for adequate assembly of β1-integrin fibrillar adhesions and demonstrated that pVHL controls the strength of cell adhesion through this mechanism. pVHL also regulates the expression of tissue inhibitors of metalloproteinases and matrix metalloproteinase 2 and 9. Their dysregulation in RCC cells with loss of pVHL allows hepatocyte growth factor/scatter factor (HGF/SF)–dependent branching morphogenesis and invasion in vitro.[40] Because epithelial-cell behaviour is clearly influenced by interaction with the ECM, disruption in the ECM may contribute to tumour invasion.

pVHL has been reported to bind directly to Jade-1,[41] Chaperonin TRiC[42] and a *VHL*-associated KRAB-A domain-containing protein (VHLak).[43] Jade-1 is a short-lived kidney-enriched transcription factor that was found to suppress renal cancer growth by promoting apoptosis in experimental studies, whereas TRiC is essential for folding *VHL* into its native functional state. VHLak, on the other hand, functions as a negative regulator of HIF-1α transactivation. pVHL is also reported to ubiquitinate proteins such as the atypical protein kinase C isotypes PKCβ and PKCλ,[44,45] and *VHL*-interacting deubiquitinating enzyme-1 and -2.[46,47] Loss of pVHL function disrupts their interactions, which may contribute to tumorigenesis.

pVHL also binds to human RNA polymerase II seventh subunit (hsRPB7), facilitating its ubiquitination and proteasomal degradation. pVHL mutation enhances hsRPB7-induced VEGF expression.[48] Recently, loss of pVHL was shown to impair p53-mediated cell-cycle arrest and apoptosis after DNA damage and to trigger the aberrant upregulation of HIF-α; this combination exerts a synergistic effect on the tumorigenesis of RCC.[49] Loss of *VHL* also enhances cyclin D1 expression,[50] whereas this loss downregulates the p27 gene in renal tumours;[51] both result in cell-cycle progression.

Genotype-phenotype correlations in VHL disease suggest that pVHL has functions independent of its role in HIF regulation. The disease of families with VHL is subdivided, based on the absence (type I) or presence (type II) of pheochromocytoma. Patients with type II pheochromocytoma are further subdivided into types by level of risk (low [IIa] or high [IIb]) of RCC, or develop only pheochromocytoma or no RCC (IIc). Families with type I disease frequently harbour *VHL*-deletion or *VHL*-truncation mutations,[52,53] whereas families with type II almost invariably harbour a *VHL* missense mutation. Patients with type I pheochromocytoma have no ability to regulate HIF, a condition that results in constitutive overexpression of HIF activity. Patients with type IIc disease retain their ability to bind and degrade HIF-α, linking the development of RCC to constitutive HIF upregulation.[54,55]

Hereditary papillary renal carcinoma

In 1994, Zbar et al[56] reported an inherited form of papillary renal carcinoma with type 1 histologic features that affect people in their fourth to sixth decade of life. HPRC is an autosomal-dominant disease that is most often bilateral and multifocal (Fig. 1).[56,57]

MET, the gene linked to HPRC, is located at chromosome 7q31.1–34 in a 27-centimorgan interval between D7S496 and D7S1837, which encodes for a transmembrane receptor tyrosine kinase for *MET*.[58]

The *MET*-receptor tyrosine kinase is the prototypic member of a subfamily of growth factor receptors[59–63] and binds to prototypic plasminogen-related growth factors, named HGF/SF.[64,65] *MET* encodes a 150-kD amino acid precursor protein that undergoes glycosylation and subsequently cleaves into an α and a β chain.[66]

Activation of the signal transduction pathway in response to HGF/SF stimulation is mediated in part by autophosphorylation of specific tyrosine residues within the intracellular region of *MET*.

Phosphorylation of T1234 and T1235 located in the activation loop of the tyrosine-kinase domain activates the intrinsic kinase activity of the receptor,[67,68] whereas phosphorylation of T1349 and T1356 in the C-terminal of *MET* activates a mutisubstrate-docking site[69] that is responsible for signal transduction.[70–72] HGF/SF stimulation and subsequent autophosphorylation results in the promotion of cell proliferation, inhibition of apoptosis, and increased motility and tubular-structure formation, termed "branching morphogenesis."[71]

Although *MET*-HGF/SF signaling clearly mediates a variety of normal cellular processes, it has also been implicated in the generation and spread of tumours.[73] *MET* is highly expressed in the kidney, and *MET*-HGF/SF signaling has been strongly implicated in the mediation of mitogenic[74,75] and morphogenic differentiation[76] in cultured kidney cells.

Several oncogenic forms of *MET* were discovered in tumour formation. One form is oncogenically activated through a missense germline mutation identified in HPRC.[58] Trisomy 7 has also been reported in HPRC and is thought to play a role in the development of multiple renal tumours.[77]

The activation of c-*MET* in HPRC was found to possess constitutive kinase activity and malignant transforming ability.[78,79] Jeffers and colleagues[78] found that the transforming activity of c-*MET* was dependent on intracellular tyrosines at positions Y6 and Y10, in addition to Y8,9 and Y14,15, which influence a variety of *MET*-mediated responses, both in vitro (transformation, mitogenicity and invasion) and in vivo (tumorigenicity and metastasis).

A study[80] has also shown that cell-surface interactions of c-*MET* in its β chain with extracellular signal-transduction molecules, such as plexin B1 and integrin α6β4, may enhance the invasiveness and metastatic potential of c-*MET* by inducing cytoskeletal changes.

In renal cancer, the *MET* and *VHL* signaling pathway is thought to intersect by means of pVHL-mediated regulation of HIF function. HIF stabilization through hypoxia or loss of *VHL* function results in overexpression of c-*MET* and forms a synergistic effect in inducing invasion.[81]

Hereditary leiomyomatosis and renal cancer

In 2001, Launonen and colleagues[82] reported a disease complex of renal cancer associated in a studied kindred that included 11 family members with uterine leiomyoma and 7 members with a history of cutaneous nodules or leiomyoma. The family members affected were in their third to fourth decade of life and the histologic type of renal can-

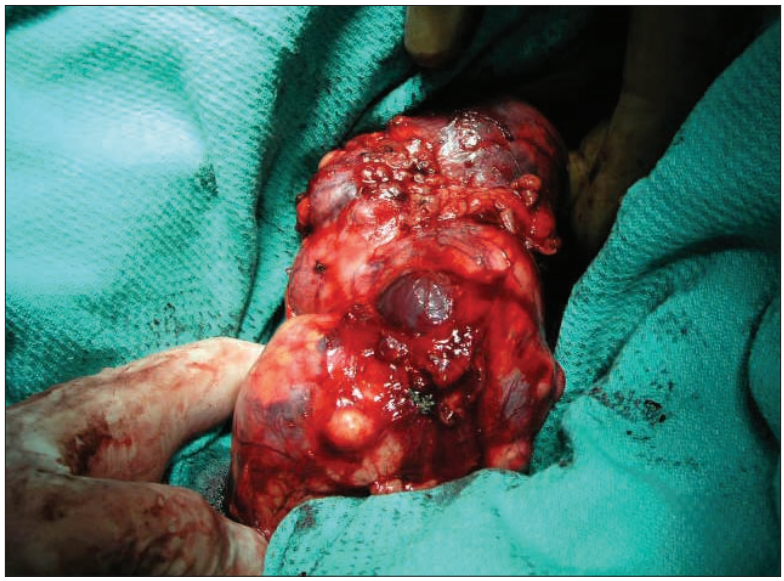

Fig. 1. A case of a 47-year-old man with bilateral multifocal papillary renal cell carcinoma. Germline testing found an A-to-G mutation at nucleotide 3529 of the *MET* proto-oncogene.

cer was papillary type 2. This autosomal-dominant syndrome[83] was later known as HLRCC. A small proportion of families with multiple cutaneous and uterine leiomyoma also have a cluster of renal collecting-duct cancer.[84,85]

The usual clinical presentation of HLRCC is the development of multiple leiomyoma in the skin and uterus. In 1 small series study,[86] the frequency of papillary type 2 renal cancer was up to 62%. Often solitary but aggressive, this cancer has an early onset. The behaviour of these tumours seems contrary to that of HPRC tumours.

In a genome-wide linkage analysis with 370 microsatellite markers, the predisposition locus of HLRC maps to chromosome 1q42–44,[82] which is caused by a germline mutation in fumarate hydratase (*FH*) in the majority of screened patients. Most of the germline mutations are missense mutations, but small deletions, insertions and nonsense mutations have been reported.[87] Molecular analysis has shown that the wild-type of the *FH* allele is lost in HLRCC-associated tumours, indicating that *FH* acts as tumour-suppressor gene.[88]

FH catalyzes the hydration of fumarate to malate as part of the tricarboxylic acid cycle in the mitochondrial matrix. Loss of *FH* activity during progressive catalysis in the mitochondria during the Krebs cycle disrupts the process, resulting in increased fumarate levels and an increased concentration of its precursor, succinate.

Although the mechanism of tumorigenesis in HLRCC remains unclear, 2 notable proposed theories may explain its tumorigenesis: pseudohypoxic drive and defective apoptosis caused by mitochondrial dysfunction or structural changes. Further research will further elucidate these mechanisms.

Birt-Hogg-Dubé syndrome

BHD syndrome was first reported in 1977 in a large kindred who had small papular lesions that originated in the hair follicles of the head and neck, appearing during the third and fourth decades of life.[89] This rare autosomal-dominant syndrome is characterized by hair follicle hamartomas, pulmonary cyst, spontaneous pneumothorax and renal cell tumours.[90]

The *BHD* gene was localized to chromosome 17p11.2,[91,92] which encodes folliculin,[90] a protein currently of unknown function. But a recent a study[93] suggested that folliculin and its interacting partner FNIP1 may be involved in the energy or nutrient pathway regulated by 5'AMP-activated protein kinase and mammalian target of rapamycin, a protein kinase that regulates cell growth, proliferation, motility and transcription.

Renal cancer, which may develop in 15%–30% of patients with BHD,[94] is usually multiple and bilateral.[95] Renal tumours in BHD usually demonstrate more than 1 coexisting histologic type, such as chromophobe (34%), oncocytoma (5%), clear-cell (9%) and papillary (1.5%) RCC, but the largest subset (65%) is a hybrid comprising oncocytoma and chromophobe RCC.[96]

In a study by Schmidt and colleagues,[97] affected members inherited an insertion or deletion of a cytosine in a C8 tract in exon 11, which represents a hypermutable hotspot for mutation in the *BHD* gene. Significantly fewer renal tumours were observed in patients with the C-deletion than those with the C-insertion mutation.

On the other hand, Vocke and colleagues[98] reported that somatic mutations in the second copy of *BHD* were distributed across the entire gene. The majority resulted in frameshifts that are predicted to truncate the BHD protein while the loss of heterozygosity at the *BHD* locus was detected in a minority of additional tumours. These results support a role for *BHD* as a tumour-suppressor gene that predisposes a person to develop renal tumours when both copies are inactivated.

Sporadic kidney cancer

Clear-cell (conventional) RCC

Clear-cell RCC accounts for approximately 75% of all sporadic forms of RCC. The origin of this cell type is thought to be from proximal tubules.[94] From a study[9] of families with VHL and linkage analysis, the *VHL* gene responsible for clear-cell RCC was identified on a small locus on the short arm of chromosome 3. This gene was found to act as a loss-of-function tumour-suppressor gene and was mutated or underwent methylation in a high proportion of tumours from patients with sporadic forms of clear-cell RCC.[99] The frequency of this gene inactivation has been reported in up to 60%–70% of these patients.[100]

Similar to the pathway found in familial disease, one allele is mutated and the other is inactivated.[99]

The difference in sporadic RCC is that an additional step is required for the development of cancer: both genetic changes must occur independently because the *VHL* gene has no inherited pre-existent abnormality. The downstream effects are identical; loss of inhibition of the *VHL* gene on HIF-1α leads to its accumulation, resulting in increased transcription of the target genes. The hypervascularity of the clear-cell RCC and other *VHL*-mediated tumours has been attributed to this overexpression.[101]

Unlike the familial form, the resulting phenotype is commonly a solitary tumour that occurs later in life. No mutations of the *VHL* gene have been found in sporadic RCC outside of the clear-cell variant.[102] To date, no phenotypic patterns have been associated with specific tumour mutations.

Papillary RCC

Papillary RCC accounts for 15% of sporadic tumours. The cells originate in the proximal tubule.[103] The 2 distinct histological subtypes are classified as type 1 and 2 papillary RCC.[104] type 1, which is rare, is associated with *MET* gene mutations, whereas type 2 has none of these mutations and is most commonly encountered in sporadic forms of RCC and more recently in HLRCC.[105]

Papillary RCC, which is morphologically and cytogenetically different from clear-cell RCC, is found 5–8 times more frequently in males than in females, and more often in the elderly.[103,106] In hereditary papillary RCC, as discussed earlier, the gene responsible is *MET* on chromosome 7, which encodes for receptor tyrosine kinase that is normally activated by HGF/SF.

An activating mutation to this gene in HPRC allows its transformation from a proto-oncogene into an oncogene, which becomes autonomous and results in eventual unregulated cellular proliferation through signal-transduction pathways.[107] Chromosome 7 is then duplicated, increasing its level of expression and producing greater numbers of protein copies. However, this activated mutation of the *MET* gene is found in only 13% of cases in its sporadic counterpart.[58,108]

Changes that were mostly found in sporadic papillary tumours were 2- or 3-fold gains of wild-type chromosome 7.[109] It is postulated that these gains may be sufficient to result in tumour formation without an activating mutation in the *MET* gene.[109] These gains are found in up to 80% of sporadic papillary forms of RCC, along with duplications of chromosome 17 or loss of the Y chromosome.[110]

A few cases of sporadic forms of papillary RCC have been associated with translocations involving chromosome X, in which it has been fused to the *TFE3* transcription gene.[111] This transcription-encoding gene is related to the proto-oncogene *myc*; this dysregulated fusion may be the basis for RCC in this subtype.[112,113] These tumours often affect children and young adults. However, the genes responsible for the majority of cases of sporadic papillary RCC are yet to be elucidated.[113]

Chromophobe, oncocytoma and collecting-duct RCC

The incidence of chromophobe, oncocytoma and collecting-duct tumours is 4%, 4% and less than 1%, respectively.[103] No specific genes have been characterized as essential to the development of sporadic forms of these tumours.

The *BHD* gene is implicated in the rare familial autosomal-dominant condition, BHD syndrome. *BHD* mutations can occur rarely in sporadic RCC.[114,115] Further understanding of the role of alterations in the *BHD* gene remains a focus of research study today.

Molecular profiling in sporadic forms of RCC

RCC is a heterogeneous epithelial malignancy with different subtypes and tumour biology. Development of novel molecular methods may one day enhance diagnosis before treatment, which may, in turn, increase detection of incidental renal masses and the existence of its benign counterpart. Incorporation of new molecular markers into current staging systems can revolutionize the staging of RCC and provide more accurate prognostication.[116]

Molecular markers will play an important role in moving from nonspecific treatments to targeting specific therapy in local, locally advanced and metastatic RCC. Methods based on the emergence of gene arrays have made it possible to investigate the expression of large numbers of genes together. An example of this form of treatment is targeting the proteins in the hypoxia-inducible pathway in clear-cell RCC.

Conclusion

Significant developments in understanding the underlying genetic basis of RCC have occurred over the last 2 decades. These advances have been attributed to intensive research into rare inherited renal cancer syndromes and identification of the genes responsible for these syndromes. Many of the genes involved are also found in sporadic RCC.

Understanding the molecular mechanisms involved in the pathogenesis of RCC has aided the development of molecular-targeted drugs for this disease. Experimental drugs with promising results are currently targeting the components of the VEGF (angiogenesis) and transforming-growth-factor-α (growth-factor) pathway of advanced RCC. During the next decade, many more clinical trials will clarify the effectiveness of this strategy. Urologists need to ensure that their understanding of the molecular events in the development of RCC remains current because future management of RCC may belong to a different paradigm.

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