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Molecular biology of renal-cell carcinoma

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ABSTRACT

Renal-cell cancer (RCC) is an heterogeneous disease consisting of different subtypes that show peculiar histological features and genetic alterations. Although inherited or familial predisposition occurs in less than 4% of renal cancers, most of the available information on the genetic alterations involved in the pathogenesis of RCC derives from the study of the inherited forms of kidney cancer: von Hippel-Lindau (*VHL* gene), hereditary papillary renal carcinoma (*MET* proto-oncogene), hereditary leiomyomatosis and renal-cell cancer (*fumarate hydratase* gene), and Birt–Hogg–Dube (*BHD* gene) syndromes. Such genetic alterations have also been detected in sporadic RCCs. In particular, inactivation of *VHL* gene by mutation or hypermethylation has been found in up to 70% of sporadic clear-cell RCC, and it has been associated with increased hypoxia-inducible factor (HIF) activity. The knowledge of these deregulated genes and their downstream pathways provides the rationale for the development of target-based approaches for RCC.

1. Introduction

Renal-cell cancer (RCC) is an heterogeneous disease consisting of different subtypes that show peculiar histological features and genetic alterations. Clear-cell RCC is the most frequent form of renal cancer with an incidence of 75%, followed by papillary types I and II tumours (10%), chromophobe tumours (5%), carcinoma of the collecting ducts of Bellini (1%) and other rare types.[1] The genetic alterations of the RCC subtypes have been defined and extensively studied in the hereditary forms of renal tumours, although inherited or familial predisposition occurs in less than 4% of renal cancers.[1] However, the data obtained from the studies of hereditary forms led to the subsequent identification of the genes and the mechanisms involved in the pathogenesis of sporadic RCCs. The knowledge of these deregulated genes and their downstream pathways provides the rationale for the development of target-based therapeutical approaches for renal cancer.

2. The VHL gene and clear-cell renal cancer

The von Hippel-Lindau (VHL) syndrome is an inherited autosomal-dominant disease (Table 1). Individuals with the VHL syndrome are at risk to develop different cancer types, including tumours of the central nervous system, retina, adrenal glands (pheochromocytoma) and bilateral, multifocal clear-cell RCC.[2] The *VHL* tumour suppressor gene that leads to this cancer syndrome is located at chromosome 3p25-26.[3] Individuals with VHL syndrome carry one wild-type and one inactivated *VHL* allele. Tumour development in VHL disease is associated with somatic inactivation or loss of the remaining wild-type *VHL* allele. *VHL* mutations are extremely heterogeneous, i.e. large or partial germline deletions, missense or nonsense or frameshift mutations have been detected.[4] In total, more than 150 different germline *VHL* mutations associated with VHL disease have been reported.[4] Clinically, VHL patients were subdivided into different subtypes, with respect to the correlation between *VHL* mutations and the risk of development of different VHL-

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Table 1 – Heritable syndromes most frequently associated with renal-cell carcinoma

Inherited disease	Gene	Locus	RCC subtype
Von Hippel-Lindau disease (VHL)	VHL	3p25	Clear-cell RCC
Hereditary papillary renal cancer (HPRC)	MET	7q31	Type-1 papillary RCC
Hereditary leiomyomatosis and renal-cell cancer (HLRCC)	FH	1q42	Type-2 papillary RCC
Birt–Hogg–Dubè syndrome (BHD)	BHD	17p11	Chromophobe RCC hybrid oncocytic RCC clear-cell RCC oncocytoma

associated tumours. In particular, type-1 families have nonsense *VHL* mutations and deletions, which cause total loss of VHL function and predispose to the entire spectrum of VHL-syndrome manifestations, except to pheochromocytoma. Type-2 families almost invariably have missense *VHL* mutations that reduce VHL function and predispose to all the different manifestations of VHL syndrome, including pheochromocytoma with or without RCC. Type-2 families have been subdivided into type-2A (low risk of RCC), type-2B (high risk of RCC) and type-2C (familial pheochromocytoma without RCC). *VHL* alleles linked to types-1, -2A and -2B VHL disease causing RCCs encode proteins that are at least partially defective with respect to hypoxia-inducible factor (HIF) regulation, whereas the proteins associated with type-2C disease show normal ability to regulate HIF, but lack other functions of pVHL.[5,6] These findings suggest that the VHL–HIF interaction plays a fundamental role in RCC pathogenesis.

Biallelic *VHL* inactivation is involved in the pathogenesis of sporadic clear-cell RCC. In fact, somatic mutations of the *VHL* gene have been detected in approximately 50% of sporadic clear-cell carcinoma. However, hypermethylation that leads to functional inactivation of the gene has also been observed in 10–20% of sporadic clear-cell RCC. Therefore, inactivation of the VHL pathway is involved in up to 70% of sporadic clear-cell carcinoma, whereas alterations of the *VHL* gene are rarely detected in other histologic subtypes.[4]

The *VHL* gene product, pVHL, is part of an intracellular multiprotein complex that contains elongin C and B, Cul2 and Rbx1 and functions as the substrate recognition component of an E3-ubiquitin ligase.[7] This complex selects specific substrates for ubiquitination and targets them for destruction through the proteasome. Among the different targets, pVHL is able to induce proteosomal degradation of HIF. This protein is an heterodimer consisting of unstable α-subunits and stable constitutively expressed β-subunits. There are three HIF-α genes in human genome, HIF-1α and HIF-2α, which activate the transcription in mammalian cells and HIF-3α, whose splice variants, such as HIF-3α4, act as dominant-negative inhibitors of HIF activity.[5,8] In normoxia, the α-subunits are the target for prolyl hydroxylation that generates a binding site for pVHL. The interaction between the two proteins causes the polyubiquitynation and subsequent proteosomal degradation of HIF-α.[5] Maxwell et al., demonstrated the key role for pVHL in the HIF regulation. In cells with normal VHL function, the α subunits of HIF are rapidly degraded by the proteasome whereas in VHL-defective cells HIF-α subunits are constitutively stabilized.[9] Therefore, the absence of pVHL mimics hypoxia and results in a constitutive up-regulation of HIF that produces a transcriptional activation of several genes involved in tumour growth and angiogenesis, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor-β (PDGF-β), transforming growth factor-α (TGF-α), erythropoietin and glucose-transporter-1 (GLUT-1). In particular, the enhanced angiogenesis appears as a fundamental phenomenon in the pathogenesis and progression of this type of cancer. Recently, it has been shown that VEGF protein expression in *VHL*-defective renal cancer cells is mainly induced by HIF-2α activity.[10] The expression of other HIF targets, such as GLUT-1, urokinase-type plasminogen activator receptor (uPAR), and plasminogen activator inhibitor-1 (PAI-1), was also regulated by HIF-2α in these cells.[10] Taken together, these data suggest a key role of HIF-2α in tumourigenesis mediated by loss of *VHL*.

It is important to underline that growth factors up-regulated by HIF, such as VEGF and TGF-α, might bind to the respective tyrosine kinase receptors (EGFR and VEGFR-1) that are expressed in renal cancer cells.[11,12] Tyrosine kinase receptors trigger activation of signal transduction pathways involved in cell proliferation and survival of RCCs.[13] In this respect, constitutive activation of the Raf/MEK/Erk pathway has been demonstrated in approximately 50% of RCC samples.[14]

In further studies, it has been shown that *VHL* loss drives nuclear factor-kB (NF-kB) activation, resulting in accumulation of HIF-α.[15] In contrast, Sourbier et al., found that intrinsic resistance of RCCs to cell apoptosis converges on NF-kB independently on the VHL status, thus suggesting that NF-kB activity might be regulated in a VHL-independent manner in RCC cells.[16] Thus additional experiments focusing on that particular point are needed to more precisely define the role, if any, of the *VHL* gene in the regulation of NF-kB during RCC tumourigenesis.

3. The MET proto-oncogene and type-1 papillary renal cancer

Individuals with hereditary papillary renal cancer syndrome (HPRC) are at risk to develop multifocal, bilateral type-1 papillary RCC (Table 1).[2] This cancer syndrome is inherited with an autosomal-dominant pattern. The disease locus was mapped on chromosome 7q31, where the *MET* proto-oncogene is located.[17] The *MET* proto-oncogene encodes a receptor tyrosine kinase that is physiologically activated by hepatocyte growth factor/scatter factor (HFG/SF). Binding of HFG/SF to the extracellular portion of the MET receptor triggers autophosphorylation of critical tyrosines in the intracellular tyrosine kinase domain, thus activating a downstream signalling cascade.[18] Activation of the MET/HGF signalling pathway has been shown to be involved in a number of biological activities

including cell proliferation, cell motility, branching morphogenesis and epithelial-mesenchymal transition.[18,19]

Mutations of the *MET* proto-oncogene were detected in germline of HPRC patients and in a subset of sporadic papillary renal carcinomas. All mutations were missense mutations and were located in the tyrosine kinase domain of *MET*.[17] Trisomy of chromosome 7 with duplication of the chromosome carrying the mutated *MET* gene is characteristic of type-1 papillary RCCs, suggesting that such duplication might represent a mechanism of further oncogene activation. In this regard, 95% of sporadic papillary renal carcinomas also showed trisomy of chromosome 7.[20] Otherwise, in sporadic type-1 papillary RCCs, *MET* mutations were observed only in a 13% of patients.[21] These observations indicate that tumorigenesis of most sporadic papillary RCCs are not be induced only by *MET* mutations, but mostly by *MET* and chromosome 7-related genes dosage.

Since the frequency of *MET* mutations in sporadic papillary RCCs is low, it is possible that other genes might be involved in this form of renal cancer. In this respect, mutations of the *KIT* proto-oncogene have been described in 68% of papillary RCC patients (Catalogue of Somatic Mutations, release 37, Wellcome Trust Sanger Institute). A recent paper by Lin *et al.* confirmed these findings.[22] However, the role of KIT in the pathogenesis of this disease needs to be confirmed by functional studies.

4. The fumarate hydratase gene and type-2 papillary renal cancer

Hereditary leiomyomatosis and renal-cell cancer (HLRCC) is an autosomal-dominant cancer syndrome (Table 1). Individuals with HLRCC have an increased risk to develop cutaneous and uterine leiomyomas, uterine leiomyosarcoma and solitary, unilateral type-2 papillary RCCs. Type-2 papillary RCC is an aggressive cancer that differs from type-1 in morphologic and cytogenetic features.[1,23] In fact, papillary RCC type-1 is characterised by cells with scant cytoplasm that are arranged in single layers on papillary cores and often contain foamy macrophages, whereas papillary RCC type-2 is characterised by larger cells with eosinophilic cytoplasm and pseudostratified nuclei.[24]

The gene predisposing to HLRCC was located on chromosome 1q42–43[25], and later identified as the fumarate hydratase gene (*FH*).[26] Different mutations have been identified in *FH* that predispose to cutaneous and uterine leiomyomas and renal cancer.[6] *FH* mutations include protein-truncating mutation, large germline deletions, missense changes and an in frame deletion that all produce severe reduction in enzyme activity.[26] Loss of the wild-type allele and acquired somatic mutations have been observed in papillary RCC from patients with HLRCC, thus indicating that *FH* acts as a tumour suppressor gene.[6] In sporadic tumours mutations of the *FH* gene appear to be extremely rare.[27]

With respect to the pathogenesis of renal cancer in FH-deficient patients, it has been proposed that the inactivation of this enzyme might lead to an hypoxic environment that can favour renal carcinogenesis. In fact, FH is an enzyme component of the mitochondrial tricarboxylic acid or Krebs Cycle that has an important role in energy metabolism. FH-inactivating mutations increase fumarate levels, and consequently the concentration of the fumarate precursor succinate. The high levels of succinate in the cytoplasm lead to stabilization of HIF-1α subunits and transcriptional upregulation of hypoxia-inducible genes.[7]

More recently, a role of *MYC* gene in the pathogenesis of papillary type-2 RCC has been hypothesised. In particular, Furge et al. established that type-2 papillary RCC is associated with amplification of chromosome 8q and subsequent overexpression of the *MYC* gene, that leads to activation of MYC signalling.[28]

5. The Birt–Hogg–Dubè gene and renal carcinoma

The Birt–Hogg–Dubè (BHD) syndrome is an autosomal-dominant disease (Table 1). Affected individuals are at risk to develop fibrofolliculomas, spontaneous pneumotorax, lung cysts and RCCs, in particular chromophobe RCC (33%), hybrid oncocytic renal-cell carcinoma (50%), clear-cell RCC (9%) and oncocytomas (5%).[2] The *BHD* locus was mapped on chromosome 17p11.2.[29,30] The *BHD* gene encodes for a protein called folliculin, which has no significant homology to any known human protein, although it is highly conserved across the species.[31] All germline *BHD* mutations are insertions, deletions, nonsense and splice-site mutations that predict truncation of the protein.[32] In addition, somatic mutations in the second copy of *BHD* or loss of heterozygosity at the *BHD* locus were observed in 70% of patients with germline *BHD* mutations, thus suggesting a role for *BHD* as tumour suppressor gene.[33] In sporadic cases, somatic *BHD* mutations are rare, but *BHD* promoter methylation has been observed, indicating the involvement of *BHD* in sporadic RCC tumorigenesis.[34]

The function of folliculin has not been completely elucidated yet. Recently, folliculin has been shown to interact with FNIP1, which binds to the 5′ AMP-activated protein kinase (AMPK), an energy sensor that negatively regulates mammalian target of rapamycin (mTOR).[35] Therefore, these findings suggest a potential role of folliculin in regulating the activation of this pathway that is involved in cell survival.

Finally, recent papers have shown the involvement of *TP53* in RCCs. Interestingly, the *TP53* gene is located at 17p13.1, near *BDH*. *TP53* mutations occur preferentially in chromophobe RCCs, suggesting a specific role of TP53 in this subtype.[36]

6. Conclusions

Identification of the genes deregulated in inherited and in sporadic forms of renal cancer allowed the understanding of the molecular basis of the different subtypes and the developing of therapies against specific targets involved in the altered signalling pathways. In particular, the agents that target HIF-up-regulated factors and pathways are in clinical development or have been approved for the treatment of clear-cell RCCs. Deregulated HIF pathway is also associated with *FH* mutations. Therefore, the agents used for the treatment of

clear-cell RCC might be effective for inherited type-2 papillary RCC. Inhibitors against the wild-type or some mutant forms of *MET* are currently under investigations. Finally, recent findings on *BHD* pathway should provide the rationale for the development of therapy for the different subtypes, carrying *BHD* mutations.

Conflict of interest statement

The authors disclose no financial and personal relationship with other people or organizations that could inappropriately influence their work.

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REFERENCES

1. Lopez-Beltran A, Scarpelli M, Montironi R, et al. 2004 WHO classification of the renal tumors of the adults. *Eur Urol* 2006;**49**:798–805.
2. Linehan WM, Pinto PA, Srinivasan R, et al. Identification of the genes for kidney cancer: opportunity for disease-specific targeted therapeutics. *Clin Cancer Res* 2007;**13**:671s–9s.
3. Latif F, Tory K, Gnarra J, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 1993;**260**:1317–20.
4. Kim WY, Kaelin WG. Role of VHL gene mutation in human cancer. *J Clin Oncol* 2004;**22**:4991–5004.
5. Kaelin Jr WG. The von Hippel-Lindau tumor suppressor gene and kidney cancer. *Clin Cancer Res* 2004;**10**:6290S–5S.
6. Pavlovich CP, Schmidt LS. Searching for the hereditary causes of renal-cell carcinoma. *Nat Rev Cancer* 2004;**4**:381–93.
7. Iliopoulos O. Molecular biology of renal cell cancer and the identification of therapeutic targets. *J Clin Oncol* 2006;**24**:5593–600.
8. Maynard MA, Evans AJ, Shi W, et al. Dominant-negative HIF-3 alpha 4 suppresses VHL-null renal cell carcinoma progression. *Cell Cycle* 2007;**6**:2810–6.
9. Maxwell PH, Wiesener MS, Chang GW, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 1999;**399**:271–5.
10. Carroll VA, Ashcroft M. Role of hypoxia-inducible factor (HIF)-1alpha versus HIF-2alpha in the regulation of HIF target genes in response to hypoxia, insulin-like growth factor-I, or loss of von Hippel-Lindau function: implications for targeting the HIF pathway. *Cancer Res* 2006;**66**:6264–70.
11. Rivet J, Mourah S, Murata H, et al. VEGF and VEGFR-1 are coexpressed by epithelial and stromal cells of renal cell carcinoma. *Cancer* 2008;**112**:433–42.
12. Normanno N, Bianco C, De Luca A, et al. Target-based agents against ErbB receptors and their ligands: a novel approach to cancer treatment. *Endocr Relat Cancer* 2003;**10**:1–21.
13. Patel PH, Chadalavada RS, Chaganti RS, et al. Targeting von Hippel-Lindau pathway in renal cell carcinoma. *Clin Cancer Res* 2006;**12**:7215–20.
14. Oka H, Chatani Y, Hoshino R, et al. Constitutive activation of mitogen-activated protein (MAP) kinases in human renal cell carcinoma. *Cancer Res* 1995;**55**:4182–7.
15. An J, Rettig MB. Mechanism of von Hippel-Lindau Protein-Mediated Suppression of Nuclear Factor kappa B Activity. *Mol. Cell. Biol.* 2005;**25**:7546–56.
16. Sourbier C, Danilin S, Lindner V, et al. Targeting the nuclear factor-kappaB rescue pathway has promising future in human renal cell carcinoma therapy. *Cancer Res* 2007;**67**:11668–76.
17. Schmidt L, Duh FM, Chen F, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. *Nat Genet* 1997;**16**:68–73.
18. Birchmeier C, Birchmeier W, Gherardi E, et al. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003;**4**:915–25.
19. Boccaccio C, Comoglio PM. Invasive growth: a MET-driven genetic programme for cancer and stem cells. *Nat Rev Cancer* 2006;**6**:637–45.
20. Kovacs G. Molecular cytogenetics of renal cell tumors. *Adv Cancer Res* 1993;**62**:89–124.
21. Schmidt L, Junker K, Nakaigawa N, et al. Novel mutations of the MET proto-oncogene in papillary renal carcinomas. *Oncogene* 1999;**18**:2343–50.
22. Lin ZH, Han EM, Lee ES, et al. A distinct expression pattern and point mutation of c-kit in papillary renal cell carcinomas. *Mod Pathol* 2004;**17**:611–6.
23. Jiang F, Richter J, Schraml P, et al. Chromosomal imbalances in papillary renal cell carcinoma: genetic differences between histological subtypes. *Am J Pathol* 1998;**153**:1467–73.
24. Delahunt B, Eble JN, McCredie MR, et al. Morphologic typing of papillary renal cell carcinoma: comparison of growth kinetics and patient survival in 66 cases. *Hum Pathol* 2001;**32**:590–5.
25. Alam NA, Bevan S, Churchman M, et al. Localization of a gene (MCUL1) for multiple cutaneous leiomyomata and uterine fibroids to chromosome 1q42 3-q43. *Am J Hum Genet* 2001;**68**:1264–9.
26. Tomlinson IP, Alam NA, Rowan AJ, et al. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat Genet* 2002;**30**:406–10.
27. Kiuru M, Lehtonen R, Arola J, et al. Few FH mutations in sporadic counterparts of tumor types observed in hereditary leiomyomatosis and renal cell cancer families. *Cancer Res* 2002;**62**:4554–7.
28. Furge KA, Chen J, Koeman J, et al. Detection of DNA copy number changes and oncogenic signaling abnormalities from gene expression data reveals MYC activation in high-grade papillary renal cell carcinoma. *Cancer Res* 2007;**67**:3171–6.
29. Khoo SK, Bradley M, Wong FK, et al. Birt–Hogg–Dube syndrome: mapping of a novel hereditary neoplasia gene to chromosome 17p12-q11.2. *Oncogene* 2001;**20**:5239–42.
30. Schmidt LS, Warren MB, Nickerson ML, et al. Birt–Hogg–Dube syndrome, a genodermatosis associated with spontaneous pneumothorax and kidney neoplasia, maps to chromosome 17p11.2. *Am J Hum Genet* 2001;**69**:876–82.
31. Nickerson ML, Warren MB, Toro JR, et al. Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt–Hogg–Dube syndrome. *Cancer Cell* 2002;**2**:157–64.
32. Schmidt LS, Nickerson ML, Warren MB, et al. Germline BHD-mutation spectrum and phenotype analysis of a large cohort of families with Birt–Hogg–Dube syndrome. *Am J Hum Genet* 2005;**76**:1023–33.

33. Vocke CD, Yang Y, Pavlovich CP, et al. High frequency of somatic frameshift BHD gene mutations in Birt–Hogg–Dube-associated renal tumors. *J Natl Cancer Inst* 2005;**97**:931–5.
34. Khoo SK, Kahnoski K, Sugimura J, et al. Inactivation of BHD in sporadic renal tumors. *Cancer Res* 2003;**63**:4583–7.
35. Baba M, Hong SB, Sharma N, et al. Folliculin encoded by the BHD gene interacts with a binding protein, FNIP1, and AMPK, and is involved in AMPK and mTOR signaling. *Proc Natl Acad Sci U S A* 2006;**103**:15552–7.
36. Gad S, Lefevre SH, Khoo SK, et al. Mutations in BHD and TP53 genes, but not in HNF1beta gene, in a large series of sporadic chromophobe renal cell carcinoma. *Br J Cancer* 2007;**96**:336–40.