THE VON HIPPEL-LINDAU TUMOR SUPPRESSOR PROTEIN AND KIDNEY CANCER

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Abstract Inactivation of the von Hippel-Lindau tumor suppressor gene (VHL) plays a causal role in the development of hereditary (von Hippel-Lindau disease) and sporadic clear cell carcinoma of the kidney. The VHL gene product, pVHL, is the substrate recognition subunit of a ubiquitin ligase that targets the alpha subunits of the heterodimeric transcription factor HIF (Hypoxia-inducible Factor) for destruction when oxygen is present. Cells lacking functional pVHL, or exposed to low oxygen (hypoxia), accumulate HIF, which activates a suite of genes involved in acute or chronic adaptation to hypoxia. A number of these genes, including VEGF, PDGF B, and TGF?, have been implicated in tumorigenesis. Downregulation of HIF is both necessary and sufficient for pVHL to suppress the growth of VHL-/- tumor cells in animal models, suggesting that drugs that inhibit HIF, or HIF-responsive gene products, might be useful for the treatment of clear cell kidney cancer. Indeed, multiple drugs that inhibit VEGF or its receptor KDR have now demonstrated activity against this disease. The rate of HIF transcription and synthesis is sensitive to changes in the activity of the PI3K-AKT-mTOR pathway. A recent randomized trial showed that patients with kidney cancer benefited from treatment with an mTOR inhibitor. Current studies are aimed at combining VEGF inhibitors with mTOR inhibitors as well as at identifying additional agents that can, at least indirectly, inhibit HIF or cancer-relevant HIF-responsive gene products.

Key words: renal carcinoma, VHl tumor suppressor gene

Resumen ***La proteína von Hippel-Lindau supresora de tumor y el cáncer renal.*** La inactivación del gen supresor von Hippel-Lindau (VHL) juega un papel determinante en el desarrollo del carcinoma hereditario (enfermedad de von Hippel-Lindau) y del carcinoma renal esporádico de células claras renal. El producto del gen VHL, pVHL, es el sitio de reconocimiento de una ubiquitina ligasa que tiene como blanco las subunidades alfa del factor de transcripción heterodimérico HIF (factor inducible por hipoxia) para su destrucción en presencia de oxígeno. Células que carecen de un pVHL funcional o que están en condiciones de baja concentración de oxígeno (hipoxia) acumulan HIF que activa una batería de genes involucrados en la adaptación aguda o crónica a la hipoxia. Algunos de estos genes, como el VEGF, PDGF B y el TGFβ han sido implicados en la tumorigénesis. La disminución de HIF es tanto necesaria como suficiente para que pVHL suprima el crecimiento de células tumorales VHL-/- en modelos animales, sugiriendo que drogas que inhiban HIF, o genes de respuesta al HIF, podrían ser útiles para el tratamiento del cáncer renal de células claras. Cabe destacar que varias drogas que inhiben VEGF o su receptor KDR han demostrado actividad contra esta enfermedad. La velocidad de transcripción y de síntesis de HIF es sensible a cambios en la actividad de la vía PI3K-AKT-mTOR. Un ensayo randomizado reciente demostró que pacientes con cáncer renal se beneficiaron al ser tratados con un inhibidor de mTOR. En la actualidad se realizan estudios dirigidos a combinar inhibidores de VEGF y de mTOR así como también a identificar agentes adicionales que puedan, indirectamente, inhibir HIF o genes respondedores a HIF que sean relevantes al cáncer.

Palabras clave: cáncer renal, gen supresor de cáncer VHL

Von Hippel-Lindau (VHL) disease is an autosomal dominant cancer syndrome that is characterized by an increased risk of multiple types of tumors including central nervous system and retinal hemangioblastomas, clear cell renal carcinomas, adrenal paragangliomas (pheochromocytomas), pancreatic islet cell tumors, and endolymphatic sac tumors[1, 2]. Individuals with VHL disease harbor a defective *VHL* tumor suppressor gene, which resides on chromosome 3p25, in their germline[3, 4]. Pathology develops when the remaining wild-type *VHL* allele is mutated or lost in a susceptible cell. Hemangioblastomas and renal cell carcinomas are the two leading cause of death in this patient population[1].

Strong genotype-phenotype correlations exist in VHL disease[2]. Type 1 families are at low risk of pheochromocy-

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toma and Type 2 families a high risk. Type 2 families can be further subdivided in Type 2A (low risk of renal cancer), Type 2B (high risk of renal cancer), and Type 2C (pheochromocytoma only). The biochemical underpinnings of these genotype-phenotype correlations are beginning to come into view (see also below).

The *VHL* gene consists of 3 exons and is ubiquitously expressed. It directs the synthesis of 2 proteins due to the use of an alternative, in-frame, translation initiation codon. The longer VHL gene product contains 213 amino acid residues and the shorter form, lacking the first 53 amino acid residues, contains 160. For simplicity, both forms will be referred to as 'pVHL' in this review because both isoforms behave similarly in most of the assays conducted to date.

As would be expected based on the study of VHL disease, *VHL* inactivation also plays a prominent role in non-hereditary clear cell renal carcinoma, which is the most common form of kidney cancer. Somatic *VHL* mutations leading to biallelic *VHL* inactivation have been reported in ~50% of sporadic clear cell renal cancers[5]. In some of the remaining tumors the *VHL* gene is not expressed because the *VHL* locus is hypermethylated[5]. The remainder of this review will focus on the role of the *VHL* gene in kidney cancer.

pVHL, HIF and Kidney Cancer

The best documented function of pVHL relates to a heterodimeric transcription factor called HIF (hypoxia-inducible factor), which consists of an unstable alpha subunit and a stable beta subunit. The human genome contains 3 HIFα genes called HIF1α, HIF2α, and HIF3α. 'HIFα' will be used in this review when referring the three family members generically. In the presence of oxygen HIFα is hydroxylated on one (or both) of two prolyl residues, which then creates a docking site for pVHL[6-10]. Hydroxylation of HIFα is mediated by members of the EglN family, especially EglN1 (also called PHD2)[11-14]. pVHL is the substrate recognition subunit of a ubiquitin ligase complex that also contains elongin B, elongin C, Cul2, and Rbx1[2]. The 3-dimensional structure of pVHL reveals two hotspots for mutations in VHL disease, called the alpha domain and the beta domain[15]. The alpha domain binds to elongin C and the remainder of the ubiquitin conjugating machinery, while the beta domain binds directly to hydroxylated HIFα.

The physical association of pVHL and HIFα leads to the polyubiquitination and proteasomal degradation of the latter. Under low oxygen (hypoxic) conditions, or in cells lacking functional pVHL, HIFα accumulates and can, in conjunction with a HIFβ family member, transcriptionally activate ~200 genes involved in acute or chronic adaptation to hypoxia including genes involved in metabolism,

angiogenesis, erythropoiesis, and cell survival[16]. Deregulation of these HIF target genes is therefore a hallmark of pVHL-defective cells.

The kidneys of VHL patients often contain numerous preneoplastic renal cysts which, when examined, are lined by *VHL*-/- epithelial cells[17-19]. It is presumed that additional mutations affecting other genetic loci are required to convert these preneoplastic lesions into invasive carcinomas. Restoration of pVHL function in *VHL*-/- sporadic clear cell renal carcinomas is sufficient to suppress their ability to form tumors in nude mice *in vivo*[20-22]. Collectively, these observations suggest that *VHL* inactivation is an early, causal, event in renal carcinogenesis and also indicate that *VHL*-/- renal carcinoma cells, despite the existence of additional mutations, remain sensitive to pVHL. In short, the *VHL* tumor suppressor gene appears to be a 'gatekeeper' for human kidney cancer.

A number of lines of evidence indicate that deregulation of HIF, especially HIF2α, contributes to the development of *VHL*-/- renal carcinomas. First, tumor suppression by pVHL can be overridden by HIF2α, but not HIF1α[23-25]. Second, *VHL*-/- renal carcinoma cells typically produce both HIF1α and HIF2α or HIF2α alone and their ability to form tumors can be blocked by selective elimination of HIF2α[24, 26]. Notably, the appearance of HIF2α in preneoplastic *VHL*-/- renal lesions coincides with histological changes indicative of increased malignancy[19]. Third, *VHL* alleles linked to a low risk of renal cancer (Type 2A and 2C) are less defective with respect to HIF regulation than are high risk alleles (Type 1 and Type 2B)[27]. Finally, HIF appears to be necessary and sufficient for the pathological changes observed in mice after *VHL* inactivation in the models examined to date[28, 29].

The apparent enhanced oncogenicity of HIF2α relative to HIF1α, at least with respect to renal cancer and possibly other tissues, probably reflects several factors. First, HIF1α, unlike HIF2α, remains relatively unstable in *VHL*-/- cells, possibly due to a second ubiquitin ligase complex[28, 30]. Second, HIF1 is more sensitive than HIF2 to regulation by FIH1[31, 32], which is an oxygen-dependent asparaginyl hydroxylase that inhibits HIFα's ability to serve as a transcriptional activator by hydroxylating a key residue involved in coactivator recruitment. Finally, it is becoming clear that the genes regulated by HIF1α and HIF2α are not entirely congruent and that these differences affect cell proliferation and survival[25, 33, 34].

Additional lines of investigation, unrelated to the *VHL* tumor suppressor gene, have also implicated HIF in the pathogenesis of kidney cancer. For example, mutations in the *TSC1* or *TSC2* tumor suppressor genes leads to the Tuberous Sclerosis hereditary cancer syndrome[35]. The products of these genes, Hamartin and Tuberin, respectively, form a complex that negatively regulates a small GTPase called Rheb. Interestingly, rats with *TSC2* mutations (Eker Rats) develop renal cancer and mice with

TSC1 or *TSC2* mutations partially phenocopy human VHL disease. Human with *TSC1* or *TSC2* mutations are also at increased risk of renal tumors although renal angiomyolipomas are much more frequent in this setting than is clear cell renal carcinoma. Nonetheless, these observations suggested that Hamartin and Tuberin might negatively regulate HIF. Indeed, cells lacking TSC1 or TSC2 overproduce HIFα and HIFα target genes such as VEGF, especially when they and their wild-type counterparts are examined under growth factor-poor conditions[36, 37]. In parallel studies, several groups showed that activation of AKT kinase, which inactivates the Hamartin/Tuberin complex, also leads to increased HIF levels in cells[38-40].

Inactivation of Hamartin/Tuberin, and activation of Rheb, leads to activation of the kinase mTOR[35]. Increased mTOR activity leads to increased HIF synthesis primarily as a result of increased HIF mRNA accumulation and HIF protein synthesis rather than to impaired HIF degradation[36]. mTOR activity can be inhibited with the small molecule rapamycin, leading to normalization of HIF levels in cells in which Hamartin/Tuberin function has been compromised[38, 40, 41].

Another potential link between HIF and kidney cancer is provided by studies of papillary renal carcinoma, which is another histological type of kidney cancer. Some cases of hereditary papillary renal cancer are due to germline, gain-of-function, c-MET alleles and some are due to germline, loss-of-function, Fumarate Hydratase Mutations[42]. The hydroxylation of HIF by EglN is obligatorily linked to the conversion of 2-oxoglutarate to succinate[43]. Succinate is converted in cells to fumarate, which is then metabolized by Fumarate Hydratase. The accumulation of fumarate or succinate can inhibit EglN function, leading to HIF accumulation[44]. Accordingly, kidney cancers linked to Fumarate Hydratase mutations display high HIF levels. It is not clear why Fumarate Hydratase mutations are linked to papillary renal carcinoma and not clear cell renal carcinoma[44]. Perhaps there is considerable 'crosstalk' between the oncogenic pathways responsible for these two types of kidney cancer. In support of this idea, several reports have suggested that pVHL loss/HIF activation leads to activation of c-Met which, as indicated above, is linked to the development of papillary cancer[45-48].

It is not clear why VHL mutations are intimately linked to kidney cancer and not other epithelial neoplasms. Several observations, however, might bear on this issue. In renal epithelial, but not other epithelial tested, pVHL inactivation leads to increased (rather than decreased) accumulation of Cyclin D1[49-51]. This appears to be due, at least partly, to establishment of an autocrine loop involving TGF and EGFR[52]. Renal epithelial, for unclear reasons, appear to be particularly sensitive to the mitogenic effect of TGFα[53].

Loss of pVHL in renal epithelium leads to loss of the primary cilium, which is a structure involved in transduc-ing mechanical signals, such as changes in flow, to mitogenesis and differentiation[54-58]. Interestingly, a number of polycystic renal diseases have in common genetic disruption of this structure. Whether loss of the primary cilium in pVHL-defective cells is HIF-dependent or not is currently disputed in the literature. Intriguing in this regard are reports that pVHL can bind directly to, and stabilize, microtubules[59]. There is also evidence that pVHL loss, possibly through HIF, leads to transcriptional downregulation of E-Cadherin and promotes and epithelial-mesenchymal transition in renal epithelial cells[60-62].

Kidney Cancer Therapeutics

The above considerations suggest that drugs that inhibit HIF activity might be effective in the treatment of kidney cancer. Unfortunately, DNA-binding transcription factors, with the exception of the steroid hormone receptors, have traditionally been difficult to inhibit with drugs. This is because the surface areas involved in DNA-binding and in dimerization, for example, are very large and do not lend themselves to disruption with small organic molecules with drug-like properties.

Fortunately, however, there are a number of drugs available that are capable of indirectly downregulating HIF or that are capable of inhibiting HIF-responsive gene products implicated in tumorigenesis. With respect to the former, mTOR inhibitors (see also above) have now been demonstrated to delay progression, and improve survival, in patients with metastatic kidney cancer in a Phase III study[63]. Interestingly, in this study patients with non-clear cell histology appeared to derive the greatest benefit. This might reflect the potential role of HIF in papillary renal cancer, as described above, or that HIF downregulation is not responsible for the clinical benefits observed in man.

Amongst HIF target genes, VEGF and PDGF have drawn particular attention because of their prominent role in tumor angiogenesis and because of the availability of inhibitory drugs. A number of drugs that inhibit VEGF, or its receptor KDR, have now demonstrated activity in the treatment of human kidney cancer. Fortuitously, a number of the KDR inhibitors currently available, including sorafenib and sunitinib, also inhibit the PDGF receptor (PDGFR), which is structurally similar to KDR. Sorafenib and sunitinib have now been approved in the U.S. for the treatment of kidney cancer based on positive results in Phase III studies[64, 65]. Both of these agent significantly delay time to progression in this setting. Whether they also improve survival is not yet clear, in part because patients who were randomized to the control treatments in these studies were allowed to crossover to these agents at the time of progression.

Conclusions

Inactivation of the *VHL* tumor suppressor gene plays a critical role in the development of human kidney cancer. The *VHL* gene product, pVHL, is part of a ubiquitin ligase complex that targets the HIF transcription factor for destruction. Deregulation of HIF target genes promotes renal tumorigenesis and drugs that downregulate HIF, or that inhibit the products of HIF target genes such as VEGF, have been shown to change the natural history of this disease. Current clinical trials are testing combinations of such agents in an attempt to improve upon these results.

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