The von Hippel-Lindau Tumor Suppressor Protein and Clear Cell Renal Carcinoma

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Abstract Germ line VHL tumor suppressor gene loss-of-function mutations cause von Hippel-Lindau disease, which is associated with an increased risk of central nervous system hemangioblastomas, clear cell renal carcinomas, and pheochromocytomas. Somatic VHL mutations are also common in sporadic clear cell renal carcinomas. The VHL gene product, pVHL, is part of a ubiquitin ligase complex that targets the α-subunits of the heterodimeric transcription factor hypoxia-inducible factor (HIF) for polyubiquitylation, and hence, proteasomal degradation, when oxygen is available. pVHL-defective clear cell renal carcinomas overproduce a variety of mRNAs that are under the control of HIF, including the mRNAs that encode vascular endothelial growth factor, platelet-derived growth factor B, and transforming growth factor α. In preclinical models, down-regulation of HIF-α, especially HIF-2α, is both necessary and sufficient for renal tumor suppression by pVHL. These observations are probably relevant to the demonstrated clinical activity of vascular endothelial growth factor antagonists in clear cell renal carcinoma and form a foundation for the testing of additional agents that inhibit HIF, or HIF-responsive gene products, in this disease.

Inactivation of the von Hippel-Lindau tumor suppressor gene (VHL), which is located on chromosome 3p25, plays an important role in hereditary (VHL disease) and sporadic clear cell renal carcinoma, which is the most common form of kidney cancer (1). The VHL gene product, pVHL, has multiple functions, but the one that has been most extensively studied relates to the regulation of the transcription factor called hypoxia-inducible factor (HIF). HIF is a heterodimer that consists of an unstable α-subunit (such as HIF-1α) and a stable β-subunit (such as HIF-1β), also called ARNT1. There are three HIF-α genes in the human genome, although only HIF1-α and HIF2-α have been well documented to activate transcription in mammalian cells under physiologic conditions. In fact, some HIF3-α splice variants probably act as dominant-negative inhibitors of HIF activity (2–4). In the presence of oxygen, HIF-α (“HIF-α”) will be used in the remainder of this review when referring to the three HIFα family members generically) subunits become hydroxylated on one (or both) of two prolyl residues (5–9). Hydroxylation of either site, which is carried out by members of the EglN family (also called PHDs or HPHs; refs. 10–12), generates a binding site for pVHL. pVHL is the substrate recognition component of a multisubunit ubiquitin ligase complex that also contains elongin B, elongin C, Cul2, and Rbx1 (also called Roc1). pVHL directs the polyubiquitylation of HIF-α, which in turn, earmarks HIF-α for proteasomal degradation (13–16). In cells that lack functional pVHL or that are exposed to low oxygen (hypoxia), HIF-α subunits accumulate and bind to a HIF-β partner protein (17). This HIF heterodimer binds to specific DNA sequences and transcriptionally activates genes involved in acute or chronic adaptation to hypoxia, such as vascular endothelial growth factor (VEGF) and erythropoietin. Over-production of such hypoxia-inducible mRNAs is a hallmark of pVHL-defective tumors (18).

Humans carrying one wild-type VHL allele and one mutated VHL allele in their germ line develop VHL disease, which is associated with an increased risk of a variety of tumors, including central nervous system (especially cerebellum and spinal cord) hemangioblastomas and pheochromocytomas, in addition to kidney cancer (19). The risk of these different tumors is influenced by the nature of the VHL mutation. In other words, there are strong genotype-phenotype correlations in VHL disease. VHL families have been subdivided into those with a low risk of pheochromocytoma (type 1 VHL disease) and those with a high risk of pheochromocytoma (type 2 VHL disease). Type 2 families almost invariably have missense VHL mutations, whereas many different types of mutations, including nonsense and deletion VHL mutations, have been linked to type 1 VHL disease. Type 2 VHL disease has been subdivided into type 2A (low risk of renal carcinoma), type 2B (high risk of renal carcinoma), and type 2C (familial pheochromocytoma without hemangioblastoma and clear cell renal carcinoma). VHL alleles linked to type 1, type 2A, and type 2B VHL disease encode proteins that are at least partially defective with respect to HIF-α regulation, whereas the products of type 2C VHL
alleles are grossly normal with respect to HIF (20, 21). The products of type 2C VHL alleles are defective, however, with respect to another pVHL function, i.e., down-regulation of atypical protein kinase C activity (22–25). Increased atypical protein kinase C activity, and consequent up-regulation of JunB, seems to promote the survival of pheochromocytoma cells when growth factors such as nerve growth factor become limiting (22).

In VHL disease, stochastic loss of the remaining wild-type VHL allele in the kidney causes the development of renal cysts (26). The precise cell of origin in these lesions is debated but might be a distal renal tubular epithelial cell (26). Regardless of the cell of origin, it is presumed that mutations at other loci are required to convert these cysts to renal cell carcinomas. A total of 40% to 80% of sporadic clear cell renal carcinomas are linked to biallelic VHL inactivation, and in some VHL+/+ clear cell renal carcinomas, little or no VHL mRNA is produced as a result of promoter hypermethylation (1).

Restoration of wild-type pVHL function in VHL−/− renal carcinoma cells suppresses their ability to form tumors in nude mice (27, 28). Although pVHL does not grossly alter cell proliferation under standard cell culture conditions, it induces a number of phenotypes in vitro that are likely to correlate with tumor suppression in vivo. These include enhanced cell cycle exit under low serum conditions and enhanced cell differentiation in monolayer and spheroid growth assays (29–34). In particular, restoration of pVHL function in VHL−/− renal carcinoma cells is able to induce a mesenchymal to epithelial transition.

VHL−/− mice develop liver hemangiomas but do not develop the lesions typical of human VHL disease (35). Similar hepatic lesions develop after Cre recombinase–mediated inactivation of VHL in the livers of VHL flox/flox mice (35, 36). These lesions are characterized by elevated levels of HIF-α and HIF-responsive gene products such as VEGF. Importantly, these lesions do not develop in mice that lack HIF-1α, also called aryl hydrocarbon receptor nuclear translocator (ARNT1), suggesting that HIF-α function is necessary for these pathologic changes (37). In a complementary set of experiments, we found that hepatic expression of a stabilized version of HIF-2α in genetically engineered mice is sufficient to induce many of the pathologic changes attributed to pVHL loss (38).

VHL−/− embryos are not viable, and conditional, systemic, inactivation of VHL in adult mice is lethal (36, 39). Haase and coworkers recently reported that inactivation of VHL in the mouse kidney, using Cre recombinase under the control of the phosphoenolpyruvate carboxykinase promoter, caused the development of polycystic and renal cysts (40). These results, however, are complicated by the fact that the Cre transgene was also expressed in the liver. Renal pathology was not observed in a mouse in which VHL was inactivated in a systemic mosaic pattern (36).

HIF-α, especially HIF-2α, seems to play a special role with respect to VHL−/− renal carcinogenesis. First, VHL−/− renal carcinomas seem to produce both HIF-1α and HIF-2α or HIF-2α alone (17). Second, the appearance of HIIF-2α in VHL−/− preneoplastic lesions arising from VHL−/− kidneys is associated with increased dysplasia (26). Third, the elimination of HIF-2α, like the restoration of pVHL function, is sufficient to suppress VHL−/− tumor growth in vivo (41, 42).

Fourth, tumor suppression by pVHL can be overridden by HIF-2α but not by HIF-1α (41, 43–45). Finally, type 2B pVHL mutants, which are associated with a high risk of renal carcinoma, are more defective with respect to HIF-α regulation than type 2A mutants (46). Collectively, these results implicate dysregulation of HIF target genes as playing a causal role in the pathogenesis of pVHL-defective clear cell renal carcinomas.

Why pVHL and HIF, both of which are ubiquitously expressed, play such an important role in human clear cell renal carcinoma is not clear, but several observations might be relevant. First, renal epithelia seem to be particularly sensitive to the mitogenic effects of the HIF-responsive growth factor transforming growth factor α among various epithelia tested (47). Second, hypoxia and HIF cause the up-regulation of cyclin D1 (Fig. 1), which with its catalytic partners, cdk4 and cdk6, stimulates cell proliferation by directing the phosphorylation of the pRB tumor suppressor protein, in renal epithelia but not in other cell types (45, 48–50). Finally, portions of the kidney in mammals (especially the medulla) are hypoxic at rest (51). Epigenetic differences between the kidney and other organs that allow renal cells to proliferate in a hypoxic environment might also make them more susceptible to the oncogenic effects of pVHL loss/HIF activation.

Kidney cancers are notoriously resistant to standard chemotherapy and radiotherapy. It has been suggested that this resistance might be due, at least in part, to increased levels of the transcription factor nuclear factor-κB (52, 53). Loss of VHL leads to increased nuclear factor-κB activity and resistance to apoptosis (54, 55). One study suggested that increased levels of HIF-α were necessary and sufficient for the activation of nuclear factor-κB, although this work needs to be independently verified (1).

**Fig. 1.** Potential cancer drug targets linked to pVHL and HIF. pVHL inhibits the heterodimeric transcription factor HIF by targeting it for proteasomal degradation. HIF protein levels are also sensitive to changes in histone deacetylase (HDAC), heat shock protein 90 (HSP90), and mTOR activities. HIF transcriptionally activates >100 genes, including many suspected or known to play roles in tumorigenesis (solid arrows). Sorafenib and sunitinib inhibit signaling by the VEGF receptor KDR and platelet- derived growth factor receptor (PDGFR). See text for details.
corroborated (56). We have obtained evidence of a HIF-independent link between pVHL and nuclear factor \( \kappa \)B.\(^2\)

These considerations suggest that drugs which target HIF, or HIF-responsive gene products, should be effective in the treatment of renal carcinomas. Unfortunately, DNA-binding transcription factors, with the exception of the steroid hormone receptors, have proven difficult to inhibit with drug-like small organic molecules. However, a number of drugs have been identified that indirectly down-regulate HIF–\( \alpha \), including drugs that inhibit mTOR (57–61), HSP90 (62, 63), and histone deacetylases (64). These drugs clearly warrant investigation in pVHL-defective renal carcinomas. In one phase 3 trial, patients with poor prognosis renal carcinoma treated with the mTOR inhibitor, CCI-779, fared better than patients treated with IFN with respect to time to progression and overall survival (65).

HIF-responsive gene products that are suspected or known to play a role in renal tumorigenesis include VEGF, platelet-derived growth factor B, c-Met (66–68), transforming growth factor \( \alpha \) (47, 69, 70), transforming growth factor \( \beta \) (71), CXCR4 (and its ligand SDF1; refs. 72, 73), and certain matrix metalloproteinase (refs. 74, 75; Fig. 1). It is worth noting that overproduction of VEGF probably occurs very early during the development of \( \text{pVHL}^-/- \) renal cell carcinomas and therefore reduces the selection pressure to activate "collateral" angiogenic pathways (26, 76, 77). This might explain why renal cell carcinomas are the only solid tumors where agents that inhibit VEGF, or its receptor KDR, have significant activity as single agents. Indeed, two such agents (sorafenib and sunitinib) were recently approved by the Food and Drug Administration for this indication. These drugs both inhibit KDR in addition to some other receptor tyrosine kinases, including the platelet-derived growth factor receptor. This might be fortuitous because dual inhibition of VEGF and platelet-derived growth factor signaling is more effective at inducing the regression of established tumor blood vessels than is VEGF blockade alone in preclinical models (78, 79). It will be important to determine whether the VHL genotype influences the responsiveness to VEGF inhibitors, as well as the molecular basis for acquired or de novo resistance. VEGF inhibitors can now serve as a platform for building rational combinations that target additional HIF-responsive growth factors and/or incorporate drugs that down-regulate HIF itself.

Open Discussion

**Dr. Atkins:** Is something other than the hypoxia-inducible factor driving the von Hippel-Lindau wild-type clear cell renal cancer?

**Dr. Kaelin:** In most VHL wild-type renal carcinoma cell lines, it looks like HIF is regulated appropriately, meaning at least it is responsive to hypoxia. I don’t know what is driving VHL wild-type clear cell renal cancer.

**Dr. Figlin:** You said that with VHL loss, angiogenesis through the vascular endothelial growth factor pathway dominates. If that were true, wouldn’t we have seen more robust clinical activity from VEGF pathway inhibitors in the form of complete responses?

**Dr. Kaelin:** I am a believer of the preclinical work performed by Ellie Keshet in Israel and Doug Hanahan at the University of California, San Francisco, which strongly suggests that newly sprouting blood vessels, before they are properly invested with pericytes and surrounding stroma, are exquisitely sensitive to, and will regress upon, VEGF withdrawal. However, once you have a mature vessel that is properly enveloped with pericytes and stroma, VEGF inhibitors no longer cause regression; they cause stasis.

**Dr. Kwon:** Is there any thinking that HIF may be a relatively late player in tumor progression?

**Dr. Kaelin:** I cannot prove that VHL loss is an early event versus a late event in sporadic clear cell carcinoma. At least in VHL disease, it appears that VHL loss is an early event and sufficient to cause renal cysts. Therefore, your question really is why would VHL loss give rise to renal cysts? VHL loss leads to up-regulation of cyclin D1 and gives rise to transforming growth factor \( \alpha \). We know that one of the features of renal cystic diseases is increased proliferation of renal epithelial cells, which might be due to cyclin D1 or transforming growth factor–\( \alpha \). Another feature is alterations in epithelial stromal interactions, and there is a role for VHL in the regulation of the extracellular matrix. Again, it is partly related to HIF. Several groups have shown that when you inactivate VHL in a renal epithelial cell, the cell undergoes an epithelial to mesenchymal transition. You can reverse that by putting VHL back in. There are a lot of effects of VHL loss that might translate into renal cyst formation. Although you might have expected HIF deregulation to be a late event, at least in renal cancer, it can be an early event.

**Dr. Kwon:** Is there a possibility that the inadequacy of angiogenesis inhibition may be in part related to exacerbation of hypoxia of the tumor and subsequent induction of more HIF?

**Dr. Kaelin:** As the tumor expands and hypoxia occurs in the surrounding normal tissues that are being compressed, the normal host becomes a source of angiogenic growth factors. Therefore, as you treat these tumors with an angiogenesis inhibitor, it is likely that you exacerbate hypoxia.

**Dr. Kwon:** What are the driving molecules that push the inception of the tumor?

**Dr. Kaelin:** I have satisfied myself as best I can that HIF is the driver.

**Dr. Sosman:** Some recent data from Neal Rosen and his colleagues has shown that if you block mTOR, you induce a feedback that enhances Akt activity. Is that pathway important in renal cancer outside mTOR-regulating HIF?

**Dr. Kaelin:** mTOR inhibitors down-regulate HIF, at least in cell culture. I do not know whether indirect effects on AKT signaling might offset any benefits of down-regulating HIF.

**Dr. Sukhatme:** At least two reports have shown that if you introduce a nondegradable HIF1 into a tumorigenic cell line, you get a decreased growth rate, but if you introduce a dominant-negative you get even faster growth. Are you concerned about HIF inhibitors in this context or is there something peculiar about that data with HIF1-\( \alpha \) versus HIF2-\( \alpha \)?

**Dr. Kaelin:** Emerging data suggest that whether HIF1 can promote or inhibit tumor growth depends on what cell type are you looking at and in what context that cell is growing.

\(^2\) Haileng Yang and Kaelin W.G., unpublished data.
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References


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