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New cancer targets emerging from studies of the Von Hippel-Lindau tumor suppressor protein

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Abstract

Inactivation of the von Hippel-Lindau tumor suppressor protein (pVHL) causes the most common form of kidney cancer. pVHL is part of a complex that polyubiquitinates the alpha subunit of the heterodimeric transcription factor HIF. In the presence of oxygen HIF α is prolyl hydroxylated by EglN1 (also called PHD2). This modification recruits pVHL, which then targets HIF α for proteasomal degradation. In hypoxic, or pVHL-defective, cells HIF α accumulates, binds to HIF β , and transcriptionally activates genes such as VEGF. VEGF inhibitors and mTOR inhibitors, which indirectly affect HIF, are now approved for the treatment of kidney cancer. EglN1 is a 2-oxoglutarate-dependent dioxygenase. Such enzymes can be inhibited with drug-like small molecules. EglN1 inhibitors are currently being tested for the treatment of anemia. EglN2 (PHD1) and EglN3 (PHD3), which are EglNs paralogs, appear to play HIF-independent roles in cell proliferation and apoptosis, respectively, and are garnering interest as potential cancer targets. A number of JmjC-containing proteins, including RBP2 and PLU-1, are 2-oxoglutarate-dependent dioxygenases that demethylate histones. Preclinical data suggest that inhibition of RBP2 or PLU-1 would suppress tumor growth.

Keywords

cancer; tumor suppressor protein; transcription factor

Individuals bearing a germline, loss of function, mutation of the von Hippel-Lindau (*VHL*) tumor suppressor gene, which resides on chromosome 3p25, develop von Hippel-Lindau disease¹. This hereditary cancer syndrome is characterized by an increased risk of developing a number of tumors including hemangioblastomas of the retina, cerebellum, and spinal cord, clear cell renal carcinomas, pheochromocytomas, endolymphatic sac tumors, pancreatic islet cell tumors, and cyst adenomas of the broad ligament or epididymis. Tumor development in this setting is linked to inactivation or loss of the remaining wild-type *VHL* allele in a susceptible cell. Biallelic *VHL* inactivation, either due to somatic mutations or hypermethylation, is also common in sporadic clear cell renal carcinomas, which are the most common form of kidney cancer.

The *VHL* gene product, pVHL, is the substrate recognition component of an E3 ubiquitin ligase complex that, in the presence of oxygen, directly interacts with the α subunit of the heterodimeric HIF (hypoxia-inducible factor) transcription factor and orchestrates its polyubiquitination and destruction². When oxygen levels are low, or pVHL is defective, HIF α accumulates, dimerizes with its partner protein HIF β (also called the aryl hydrocarbon receptor nuclear translocator or ARNT), and transcriptionally activates 100–200 genes, many of which promote survival in a hypoxic environment including genes that promote angiogenesis, erythropoiesis, and maintenance of energy homeostasis through changes in metabolism.

Recognition of HIF α by pVHL requires that the former be hydroxylated on one (or both) of two prolyl residues by members of the EglN (also called PHD) family of prolyl hydroxylases² (Fig 1). This reaction is intrinsically oxygen-dependent because the oxygen atom of the hydroxyl group is derived from molecular oxygen and because the oxygen Kms for these enzymes are just slightly above that encountered in normal tissues^{3,4}. As a result, decrements in oxygen availability lead to decreased HIF α hydroxylation. In addition to oxygen these enzymes require ferrous iron, ascorbate, and 2-oxoglutarate (also called α -ketoglutarate). The latter is decarboxylated to succinate during the course of the hydroxylation reaction (Fig 1).

EglN1 (also called PHD2) appears to be the primary HIF prolyl hydroxylase under normal conditions, with EglN2 and EglN3 playing compensatory roles under certain conditions^{5–7}. Inactivation of EglN1 in mice leads to polycythemia resulting from overproduction of erythropoietin by the kidney and germline EglN1 mutations have been linked to familial polycythemia in man^{8–11}. During fetal life the liver is the main source of erythropoietin production, with the kidney assuming this role shortly after birth. As a result, chronic renal failure, which affects over 20 million Americans, is often associated with chronic anemia¹². We recently showed, however, that inactivation of all 3 EglN paralogs is sufficient to reactivate hepatic erythropoietin production¹³. In keeping with this, EglN inhibitory drugs can induce erythropoietin in anephric mice¹⁴ and are currently being tested for the treatment of anemia in man.

pVHL has also been linked to other cellular activities that appear to be, at least partly, HIF-independent. For example, pVHL plays roles in maintenance of the primary cilium, microtubule stability, senescence, and apoptosis^{15,16}. Many of these activities could, conceivably, contribute to the tumor suppressor activity of pVHL. Nonetheless, genotype-phenotype correlations in VHL patients suggest that deregulation of HIF contributes to the pathogenesis of clear cell renal carcinomas and hemangioblastomas (the same does not appear to be true for pheochromocytomas)^{15,16}. Moreover preclinical data as well as clinical observations, outlined below, strongly suggest that increased HIF activity plays a causal role in clear cell renal carcinoma.

There are 3 HIF α family members called HIF1 α , HIF2 α , and HIF3 α . HIF1 α is the canonical, well-studied, HIF α family member and is ubiquitously expressed. The expression of HIF2 α is more restricted and it is increasingly clear that the functions of HIF1 α and HIF2 α only partially overlap. For example, HIF1 α and HIF2 α share many, but

not all, of their transcriptional targets. In addition, the two proteins differ with respect to crosstalk with other transcription factors such as c-Myc, where HIF1 α potentiates, and HIF2 α antagonizes, c-Myc activity in certain settings^{17, 18}.

pVHL-defective clear cell renal carcinomas produce HIF2 α alone or both HIF1 α and HIF2 α ¹⁹. Overproduction of a stabilized version of HIF2 α , but not HIF1 α , can override pVHL's ability to suppress tumor growth in nude mouse xenograft assays^{20, 21}. Conversely, eliminating HIF2 α is sufficient to inhibit pVHL-defective tumor growth *in vivo*^{22, 23}. In genetically engineered mouse models the phenotypes observed after pVHL inactivation appear to be largely driven by HIF2 α ^{24–27}. Finally, the appearance of HIF2 α in preneoplastic renal lesions in VHL patients heralds malignant transformation as determined by increased signs of dysplasia²⁸. Collectively, these findings support an important role for HIF2 α in clear cell renal carcinoma.

These considerations have prompted the preclinical and clinical testing of drugs that, directly or indirectly, inhibit HIF2 α or its downstream targets for the treatment of kidney cancer. For example, human kidney cancers produce very high levels of VEGF, presumably because VEGF is a HIF target, and respond to VEGF blockade in the clinic. Indeed, four drugs that inhibit VEGF (bevacizumab) or its receptor, KDR (sunitinib, sorafenib, and pazopanib) have been approved for this indication based on positive Phase 3 trial data in metastatic kidney cancer^{29–32}. The rapamycin-like drugs (rapalogs) temsirolimus and everolimus, which inhibit the mTOR kinase, have also been approved for the treatment of kidney cancer^{33, 34}. These agents indirectly downregulate HIF α within cancer cells and probably also blunt signaling downstream of VEGF in endothelial cells. Unfortunately, rapalogs preferentially inhibit mTOR when it is part of the TORC1 complex rather than part of the TORC2 complex³⁵. A recent report suggested that HIF1 α is regulated by TORC1 whereas HIF2 α is under the control of TORC2³⁶. It will be of interest to see if second generation mTOR inhibitors that inhibit both TORC1 and TORC2 will have greater activity in kidney cancer by virtue of more effective TORC2 blockade.

Kidney cancer patients treated with VEGF inhibitors or mTOR inhibitors invariably relapse and the resistance mechanisms are poorly understood. Curative therapy for kidney cancer will almost certainly require combinations of agents that have distinct mechanisms of action and which are non-cross resistant. New targets for kidney cancer will likely be produced by several lines of investigation. For example, state of the art genomic technologies, including high density SNP arrays, expression profiling, and exon resequencing, are being used in an attempt to identify the mutations that cooperate with pVHL loss to cause kidney cancer^{37–41}. In addition targets that, when inhibited, preferentially kill pVHL-defective cells due to synthetic lethal interactions are being sought by exposing isogenic cell line pairs to collections of shRNA vectors or chemical perturbants^{42, 43}. One recent study, for example, found that pVHL-defective cells are especially sensitive to agents that induce autophagy⁴³. This finding is of special interest given the clinical activity of rapalogs, which likewise promote autophagy.

Germline mutations affecting succinate dehydrogenase (SDH) and fumarate hydratase (FH) have recently linked to cancer and are also potentially linked to HIF deregulation⁴⁴. The

former have been linked to familial paragangliomas (including pheochromocytomas) and the latter to familial leiomyomatosis (cutaneous and uterine) and papillary renal carcinoma. SDH and FH mutations lead to the accumulation of succinate and fumarate, respectively, which interfere with the activities of 2-oxoglutarate-dependent dioxygenases, including the EglNs^{45–49}. As a result, these mutations lead to inappropriate HIF activation and a state of “pseudohypoxia”. Whether HIF is simply a marker of altered metabolism in these settings, or is an actual driver, is currently being explored. For example, indirect evidence, including genotype-phenotype correlations in VHL disease, argue that HIF deregulation does not play a causal role in pheochromocytoma¹⁶. Interestingly, mutations in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) have been observed in some leukemias and brain tumors^{50–54}. These mutations result in the production of 2-hydroxyglutarate, which is also suspected of altered 2-oxoglutarate-dependent enzyme activity^{55–57}.

Enzymes are preferred targets in the pharmaceutical industry because they frequently contain clefts or pockets that can bind to drug-like small organic molecules. Indeed, many recently approved anticancer agents are small ATP-like molecules that inhibit particular kinases. The EglN prolyl hydroxylases belong to a much larger superfamily of iron and 2-oxoglutarate-dependent dioxygenases⁵⁸. These enzymes can be inhibited with drug-like molecules that interfere with either iron or 2-oxoglutarate utilization. A further exploration of the biology of these enzymes therefore seems warranted.

EglN1 (PHD2) is being explored as a potential target for anemia, as outline above. A recent study also found that haploinsufficiency for EglN1 suppressed tumor growth, apparently as a result of tumor vessel normalization⁵⁹. Therefore it is difficult to predict whether EglN1 inhibitors will have protumorigenic or antitumorigenic effects in vivo.

EglN2 and EglN3 appear to have HIF-independent functions that might be relevant to tumor growth. EglN2 (PHD1) is transcriptionally induced when estrogen receptor positive breast cancer cells are induced to proliferate with estrogen⁶⁰. We showed that EglN2, in a HIF-independent manner, indirectly controls Cyclin D1⁶¹. Cyclin D1 levels are diminished in *EglN2*–/– cells and tissues including in lactating mammary glands, which display defects reminiscent of those seen in *Cyclin D1*–/– glands⁶². Downregulation of EglN2, using shRNA technology, inhibits the proliferation of a variety of cancer cells, including breast cancer cells. This defect can be rescued by wild-type, but not catalytic-dead, EglN2 as well as by Cyclin D1 itself. These findings suggest that EglN2 inhibitors might have antitumor effects, especially in estrogen-dependent breast cancers.

EglN3 (PHD3) is induced when NGF is withdrawn from neurons and appears to be both necessary and sufficient to promote apoptosis in this setting^{63–66}. EglN3 can also, at least when overproduced, induced apoptosis in a variety of cell types, in contrast to EglN1 and EglN2, and appears to do so in a HIF-independent manner^{67, 68}. How EglN3 induces apoptosis is incompletely understood but appears to involve KIF1B β , which is a candidate tumor suppressor located at 1p36^{67, 69, 70}. Recent studies suggest that EglN3 agonists, such as 2-oxoglutarate mimetics, might be used to augment cancer cell apoptosis^{71, 72}.

A number of histone demethylases that share a JmjC domain were recently found to belong to the 2-oxoglutarate-dependent dioxygenase family^{73, 74}(Fig 2). Interestingly, a number of these appear to be overexpressed or mutated in cancer, including JARIDA, JARID1B, JARID1C, UTX, and GASC1^{37, 75–80}. JARID1A (also called RBP2 or KDM5A) was originally identified as cellular protein capable of binding to the retinoblastoma tumor suppressor protein (pRB)⁸¹. pRB can induce an acute G1/S block, promote senescence, and/or promote differentiation when reintroduced into pRB-defective tumor cells. Tumor suppression by pRB is due, at least in part, to its ability to repress E2F-dependent promoters. Surprisingly, we found that certain pRB variants (natural and engineered) that cannot bind to E2F, and are incapable of repressing transcription, can nonetheless promote senescence and differentiation⁸². Such variants, however, preserve the ability to bind to JARID1A. Moreover, elimination of JARID1A, using either siRNA or genetically engineered cells, promotes senescence, differentiation, and loss of “stemness” *in vitro*⁸³(Qin Yan and W.G.K.-unpublished data). Conversely, a recent report suggested that increased expression of JARID1A mediates some forms of anticancer drug resistance by promoting a more stem cell-like phenotype⁸⁴. Similarly, JARID1B promotes breast cancer growth *in vivo*⁸⁵ and appears to maintain a stem cell-like melanoma cell population⁸⁶.

These considerations raise the possibility that changes in the levels of oxygen and specific metabolites (such as 2-oxoglutarate) will have protean biological effects through changes in the activities of various 2-oxoglutarate-dependent dioxygenases, including the enzymes dedicated to the regulation of HIF and chromatin structure. As a corollary, drugs that inhibit specific members of this family of enzymes might ultimately prove useful in the treatment of a variety of human diseases, including cancer.

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HIF Prolyl Hydroxylase Reaction

Figure 1.

Prolyl Hydroxylation of HIF α . When oxygen is present HIF α becomes hydroxylated on one (or both) of two prolyl residues by members of the EglN (also called PHD) family in a reaction that requires ferrous iron and 2-oxoglutarate. Hydroxylation targets HIF α , which targets it for degradation, is coupled to the decarboxylation of 2-oxoglutarate to succinate.

JmjC-Histone Demethylases

Figure 2. JmjC Histone Demethylase Activity. Hydroxylation of a histone methyl group by a JmjC domain-containing histone methylase leads to histone demethylation as the hydroxylated methyl group is unstable and spontaneously given off as formaldehyde.