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Renal Cancer: Oxygen meets Metabolism

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Abstract

Over the last two decades molecular studies of inherited tumor syndromes, which are associated with the development of kidney cancer have led to the identification of genes and biochemical pathways that play key roles in the malignant transformation of renal epithelial cells. Some of these findings have broad biological impact and extend beyond renal cancer. This review's focus is on the von Hippel-Lindau (VHL)/hypoxia-inducible factor (HIF) oxygen-sensing pathway and its role in physiology, energy metabolism and tumorigenesis.

Keywords

renal cell cancer; VHL tumor suppressor; hypoxia-inducible factor; oxygen; glucose metabolism; mitochondria

Introduction

Cancers of the kidney and renal pelvis affect about 170,000 patients per year worldwide, and result in approximately 70,000 deaths annually (data for 2008, International Agency for Research on Cancer; <http://globocan.iarc.fr>). For 2011 it is estimated that about 61,000 new cases will be diagnosed and approximately 13,000 patients will die from this disease in the US [1]. Renal cell cancer (RCC) represents 2% of all cancers and about 5% of all epithelial carcinomas. The overall survival of patients with advanced disease is poor and recent progress in understanding underlying molecular mechanisms have led to the development of targeted therapies for the treatment of this disease. Morphologic criteria are used to classify renal tumors into different histological subtypes, each of which associate with certain genetic alterations that give rise to distinct molecular signatures. Clear cell RCC (ccRCC) is the most common subtype and represents about 70% of all renal tumors, 15% are chromophil RCCs, which includes papillary RCC, 5% are chromophobe RCCs and the remainder consists of collecting duct carcinomas and benign oncocytomas [2]. ccRCC and chromophil RCCs are thought to originate from the proximal renal tubule, whereas chromophobe and collecting duct carcinomas are derived from the distal nephron. However, recent studies of VHL-associated RCC have questioned current dogma regarding the histogenetic origin of ccRCC. Immunohistochemical studies of early ccRCC lesions

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suggested that von Hippel-Lindau (VHL)-associated RCCs may derive from the medullary thick ascending loop of Henle or early distal tubule [3].

Over the last two decades, major insights into the molecular underpinnings of renal tumorigenesis have come from the genetic analyses of families with hereditary benign and malignant renal tumors, which represent 1–4% of all cases [4]. This led to the identification of several predisposing genes, which include the *VHL* gene located on chromosome 3p25 (VHL disease, ccRCC), the *c-MET* proto-oncogene on chromosome 7q31 (type I papillary RCC), the fumarate hydratase (*FH*) gene located on chromosome 1q42 {Hereditary Leiomyomata and Renal Cell Carcinoma (HLRCC) syndrome, type II papillary RCC and collecting duct tumors}, the Birt-Hogg-Dubé (BHD) gene folliculin (*FLCN*) on chromosome 17p11 (chromophobe RCC, oncocytoma), and the tuberous sclerosis genes *TSC1* and *TSC2* on chromosome 9q34 and 16p13.3 respectively (mostly angiomyolipomas, rare RCC and oncocytoma) [4, 5]. More recently, mutations in succinate dehydrogenase genes (*SDHB*, *SDHC* and *SDHD*), which predispose to familial paraganglioma and pheochromocytomas, have also been detected in RCC [6, 7]. While mutations in most RCC-predisposing genes are rare in non-familial tumors [8–13], mutations in the *VHL* gene product, pVHL, are detected in the majority of sporadic ccRCCs, underscoring pVHL's central role as a gate keeper in the regulation of renal epithelial cell growth and differentiation [14]. Major targets of pVHL are hypoxia-inducible factors (HIFs) [15]. HIFs are O₂-sensitive transcription factors that mediate cellular adaptation to hypoxia, and regulate a variety of hypoxia responses, which are molecular hallmarks of RCC. These include increased angiogenic growth factor production, which augments O₂ delivery, and metabolic reprogramming of cellular glucose and energy metabolism, which improves O₂ utilization. Under normal conditions when pO₂ levels are adequate, HIFs are rapidly degraded via pVHL-mediated ubiquitylation [16]. The ability to ubiquitylate HIF is lost in RCC, where HIFs are constitutively active irrespective of pO₂ levels (pseudo-hypoxia). This places pVHL into the center of cellular O₂-sensing, and links O₂-/HIF-controlled biological processes directly to renal tumorigenesis. The critical role of HIF in renal tumor formation extends beyond VHL-associated cancer, as mutations in other RCC-predisposing genes, such as FH and SDH, inhibit HIF degradation and result in HIF activation (Figure 1). This review discusses the pVHL/HIF O₂-sensing pathway in the context of renal tumorigenesis and describes recent findings that highlight HIF's role in the reprogramming of cancer cell metabolism.

Clinical manifestations of mutated pVHL

VHL disease is a rare autosomal-dominant tumor syndrome that is characterized by the development of highly vascularized tumors in multiple organs, including ccRCC. It results from germ line mutations in the VHL tumor suppressor, pVHL. The *VHL* gene was mapped to chromosome 3 in 1988 [17], and first identified by positional cloning in 1993 [18]. Although its function was unknown initially, experimental observations that O₂-dependent gene regulation was defective in *VHL*-deficient cell lines suggested a role in O₂-sensing [19]. Subsequently Maxwell and colleagues demonstrated that pVHL was critical for targeting the α -subunit of hypoxia-inducible factor (HIF) for O₂-dependent proteolysis [20], thus providing a direct molecular link between VHL-associated tumorigenesis and molecular O₂-sensing. Up to 45% of VHL patients develop ccRCC, which frequently occurs together with other disease manifestations, including renal cysts, hemangioblastomas of the central nervous system and retina, pancreatic cysts and tumors, and pheochromocytomas [21]. VHL disease is grouped clinically into 2 subtypes depending on the presence or absence of pheochromocytoma. The likelihood of organ-specific disease manifestations, i.e. whether a VHL patient is more likely to develop RCC, hemangioblastomas, pheochromocytomas or a certain combinations of tumors, depends on where in the pVHL

protein sequence a mutation has occurred. This phenotype-genotype correlation is the result of distinct functional and biochemical properties that are associated with specific pVHL mutants [22–24]. The fact that some mutant pVHL species are still able to capture and ubiquitylate hydroxylated HIF- α , indicates that HIF-independent biological functions are likely to contribute to the pathogenesis of VHL disease. The ability to degrade HIF, however, is completely lost in ccRCC. With regard to non-familial tumors, bi-allelic *VHL* inactivation is well documented for most cases of sporadic ccRCCs, and also occurs, but less frequently, in sporadic hemangioblastomas [14].

HIF regulation by pVHL

Over the last decade it has become evident that pVHL's major function is the regulation of HIF activity through ubiquitylation (for review see [25]). This function is highly conserved across species. Either loss of *VHL* expression, as a result of gene deletion or promoter hypermethylation, or mutations that affect capture and/or ubiquitylation of HIF- α , result in constitutive HIF- α stabilization and activation of HIF-controlled biological programs irrespective of pO₂ levels.

HIFs belong to the PAS (Per-ARNT-Sim) family of heterodimeric basic helix-loop-helix transcription factors and regulate gene expression by binding to hypoxia-response elements (HREs), specific DNA recognition sequences located in hypoxia enhancer-containing regulatory regions. HIFs consist of an O₂-sensitive α -subunit, and a constitutively expressed β -subunit, also known as the arylhydrocarbon receptor nuclear translocator (ARNT) or HIF- β [15, 26]. All three α -subunits that are known, HIF-1 α , HIF-2 α and HIF-3 α , are targeted by pVHL for proteasomal degradation. Whereas HIF-1 and HIF-2 heterodimers function as transcriptional activators, the role of HIF-3 α in transcriptional activation is less clear. Splice variants of HIF-3 α have been shown to inhibit HIF-dependent transcriptional activation [27, 28]. The spectrum of HIF-1 and HIF-2-regulated genes overlaps only partially. For example, anaerobic glycolysis appears to be HIF-1-controlled [29], whereas HIF-2 is the main regulator of erythropoietin (EPO) production in vivo [30, 31]. Furthermore, there is now substantial clinical and experimental evidence that HIF-1 and HIF-2 have diverse functions with regard to VHL-associated renal tumorigenesis.

pVHL-mediated poly-ubiquitylation of HIF- α requires hydroxylation of specific proline residues (Pro402 and Pro564 in human HIF-1 α ; Pro405 and Pro531 in human HIF-2 α), which are located within its O₂-dependent degradation domain (for review see [16, 32]). Hydroxylation, which is carried out by 2-oxoglutarate-dependent dioxygenases (prolyl-4-hydroxylase domain (PHD) proteins), is necessary for its rapid proteasomal degradation under normoxic conditions and requires molecular O₂, ferrous iron and ascorbate (Figure 2). A defect in the cell's ability to ubiquitylate HIF- α , i.e. as a consequence of mutated pVHL, results in HIF- α stabilization, increased HIF transcriptional activity and up-regulation of HIF target genes, such as *vascular endothelial growth factor (VEGF)*, *pyruvate dehydrogenase kinase (PDK)-1* and *EPO* (Figure 2). Aside from HRE-based transcriptional activation, HIF- α modulates cellular signaling pathways through biochemical interaction with several non-PAS domain proteins, including the tumor suppressor protein p53, c-MYC and the Notch intracellular domain (for review see [33]) (Figure 2). A second hypoxia switch operates in the carboxy-terminal transactivation domain of HIF- α with O₂-dependent hydroxylation of asparagine residue 803 by factor inhibiting HIF (FIH) (for review see [16, 32]). Inhibition of FIH facilitates CBP/p300 recruitment, which in turn increases HIF target gene expression in *VHL*-deficient cell lines or under pronounced hypoxia [34]. pVHL species that are highly associated with RCC predisposition have completely lost their ability to capture HIF- α for degradation [23, 24]. Some mutations do not result in tumorigenesis, but lead to the development of familial polycythemia, which is caused by excessive EPO

production and bone marrow hypersensitivity to EPO [35]. Chuvash polycythemia is endemic in central European Russia and is mainly associated with a mutation in *VHL* codon 200 (C598T $\Rightarrow$ R200W), which affects HIF-2 α degradation and results in abnormal signaling through Janus kinase (JAK) 2 [36, 37]. Affected individuals are typically homozygous for this hypomorphic mutation (compound heterozygotes involving other rare mutations have been reported), and are not predisposed to the development of tumors that are typically associated with VHL disease [38]. Interestingly, evaluation of cardiopulmonary function in a small group of Chuvash patients revealed significant abnormalities in respiratory and pulmonary vascular regulation at baseline, and in response to hypoxia. Basal ventilation and pulmonary vascular tone were elevated, and increases in heart rate and ventilation as well as pulmonary vasoconstrictive responses to mild or moderate hypoxia were considerably enhanced, indicating that tight regulation of the pVHL/HIF axis is required for normal cardiopulmonary physiology [39].

HIF-dependent versus HIF-independent functions of pVHL

A molecular hallmark of ccRCC is mutated pVHL. In contrast to patients with VHL disease, who transmit germ line mutations, patients with sporadic ccRCC acquire somatic mutations in both *VHL* copies. VHL-associated ccRCCs are often preceded by multifocal and bilateral cysts, which can sometimes mimic polycystic kidney disease and can be found in up to 60% of patients with VHL disease [21, 40]. About 20% of mice that lack pVHL in the proximal renal tubule develop benign cysts in a HIF-dependent manner, but do not develop ccRCCs, suggesting that HIF acts as a cystogenic transcription factor but is not sufficient for RCC development [25]. Furthermore, the notion that HIF promotes renal cyst formation is supported by in vitro experiments with ccRCC cell lines. In these studies re-introduction of wild-type pVHL restored primary cilia in a HIF-dependent manner [41]. Cystogenesis has been associated with the loss of the primary cilium, a microtubule-based organelle, which functions as a luminal flow- and chemo-sensor on epithelial and other cells [42]. Transgenic expression HIF-2 α in the murine kidney produces renal cysts and fibrosis [43]. Furthermore, genetic inactivation of fumarate hydratase, the enzyme that converts fumarate to malate and is mutated in HLRCC, results in HIF- α stabilization and kidney cyst development in aged mice (accumulation of fumarate inhibits HIF prolyl-hydroxylation) [44]. These findings, however, would have to be reconciled with reports indicating that some aspects of VHL-associated cystogenesis may be HIF-independent (see below) [45–47].

The inability to produce ccRCC in *VHL*-deficient mice, together with cytogenetic and genome-wide sequencing data from patients, indicates that transformation of cystic epithelium into ccRCC is likely to require additional genetic events that either activate oncogenes or inactivate other tumor suppressors, and are likely to involve modifications of histones and DNA repair [25, 48]. pVHL loss and HIF- α stabilization, however, represent the earliest detectable molecular events in VHL-associated renal tumorigenesis [3], which most likely trigger cellular alterations that ultimately facilitate transformation to ccRCC. These alterations are likely to include HIF-dependent and HIF-independent loss of intercellular junctions and epithelial de-differentiation resulting from E-cadherin suppression [49–52], increased senescence [53, 54], HIF-dependent and HIF-independent alterations in p53, c-MYC or NF- κ B activity [55–59], hepatocyte growth factor (HGF) signaling [60–62], increased susceptibility to transforming growth factor (TGF)- α /epidermal growth factor (EGF) signaling [63–68], as well as modifications in extracellular matrix (ECM) turnover and re-modeling [60, 69–73].

The importance of HIF in the pathogenesis and progression of ccRCC is underscored by experimental findings and clinical studies, which demonstrated that inhibition of HIF- α translation correlated with reduced tumor growth [74], and that the expression of HIF target

CXC chemokine receptor 4 was associated with disease progression [75]. Of particular importance is the role of HIF-2, as ccRCC development may depend on de novo expression of HIF-2 α or a shift in the ratio of HIF-1 α versus HIF-2 α levels towards an increase in HIF-2 α . HIF-2 α is not detectable in non-transformed renal epithelial cells under hypoxia (for a review see [33]). A bias towards HIF-2 α expression was found in clinical ccRCC samples with confirmed *VHL* defect and in ccRCC cell lines [20, 76]. Furthermore, HIF-1 α truncating mutations have been identified in several clinical samples of ccRCC by a genome wide sequencing screen, which could indicate that a shift towards HIF-2 may provide a growth advantage under certain conditions [48, 77]. In support of the concept that HIF-2 acts as an oncogene are in vitro studies, which demonstrated that HIF-2 overrides pVHL's tumor suppressor function by regulating molecular pathways that are critical for renal cell growth, such as signaling through the TGF- α /EGF receptor pathway, cyclin D1 and the c-Myc proto-oncogene [59, 64–68, 77–82].

Although the analysis of mouse models has suggested that VHL-associated phenotypes are largely HIF-mediated [25, 83], it is well established that pVHL regulates several biological processes that do not involve HIF (Figure 2). For example, pVHL appears to control microtubule stability and cilia maintenance in a HIF-independent fashion [45–47, 84, 85]. In this context, pVHL appears to cooperate with glycogen synthase kinase (GSK)-3 β in an interlinked signaling pathway that maintains the primary cilium [45]. This notion is supported by studies in conditional knock out mice, where inhibition of the PTEN tumor suppressor, which activates PI3K-Akt signaling and inhibits GSK-3 activity, promotes urogenital cyst formation in a pVHL-defective background [86, 87]. Furthermore, an additional HIF-independent link to the cysto- and oncogenic β -catenin signaling pathway exists through Jade-1, whose stability is controlled by pVHL (pVHL suppresses β -catenin signaling through Jade-1) [88].

Other pVHL-modulated cellular processes and signaling pathways that could potentially be important for VHL-associated tumorigenesis and tumor progression include signaling through atypical protein kinase C isoforms, interaction with KRAB-A domain protein, VHLak, de-ubiquitylating enzymes, the large subunit of RNA polymerase II and the RNA-binding protein hnRNP A2 [25]. The role of these proteins in the pathogenesis of VHL-associated tumors, however, awaits more detailed analysis.

HIF controls metabolic reprogramming in RCC

Increased glucose uptake and glycolytic flux are biochemical hallmarks of RCC and are exploited diagnostically with radioactively labeled glucose analogs for tumor imaging (fluorodeoxyglucose (FDG)-positron emission tomography (PET)). The proliferation of cancer cells depends on their ability to acquire nutrients that can be used for energy, i.e. ATP production and synthesis of biomass, such as nucleotides, lipids, amino acids and macromolecules. Glucose is one of the major sources for ATP and biomass synthesis. A shift of oxidative metabolism towards glycolysis, which occurs under conditions of HIF activation (Figure 3), provides carbon and NADPH (through pentose phosphate cycle) for anabolic metabolism at the expense of efficient ATP generation [89, 90]. Whether this switch towards glycolysis increases susceptibility to mutations is unclear and needs to be investigated. However, it is very likely that metabolic reprogramming of renal cells enhances their growth response to proliferative signals, as HIF activation represents an early event in RCC pathogenesis [91].

The metabolic phenotype of VHL-RCCs is also found in non-VHL renal tumors, as signaling through several RCC-predisposing gene products converges on the pVHL/HIF axis. Fumarate hydratase, the mitochondrial enzyme that is mutated in HLRCC, is part of the

tricarboxylic acid (TCA) cycle and converts fumarate to malate. Fumarate accumulates as a result of an inactivating mutation, and inhibits HIF prolyl-hydroxylation, as does succinate, which accumulates when succinate dehydrogenase (SDH) is mutated (Figure 2). This leads to HIF- α stabilization and activation of HIF-controlled transcriptional programs. Mutations in FH and in SDH subunits predispose to renal tumors, at least partially by activating HIF, and link the TCA cycle to renal tumorigenesis (Figure 1). Other RCC-predisposing gene products, such as c-MET, TSC1, TSC2 and folliculin, signal through mammalian target of rapamycin (mTOR), which increases the translation of HIF- α [92] (Figure 1). The importance of mTOR signaling in the regulation of HIF translation is underscored by the observation that VHL-deficient RCC cells are particularly sensitive to mTOR inhibition, which has striking effects on HIF signaling, glucose metabolism and tumor growth [74].

HIF regulates cellular energy and glucose metabolism at multiple levels (Figure 3). Under hypoxic conditions when oxidative metabolism is impaired, HIF-1 shifts metabolism from oxidative phosphorylation to anaerobic glycolysis [93]. HIF-1 mediates this effect, which is also referred to as the Pasteur effect, by increasing the expression of glycolytic enzymes, such as hexokinase (HK), phosphofructokinase, aldolase, phosphoglycerate kinase 1, enolase and lactate dehydrogenase (LDH), and by blocking the conversion of pyruvate to acetyl coenzyme A (CoA). The latter occurs through transcriptional up-regulation of pyruvate dehydrogenase kinase (PDK), which inhibits pyruvate dehydrogenase, thereby uncoupling glycolysis from the tricarboxylic acid (TCA) cycle [94, 95]. As a result, mitochondrial O₂ consumption is decreased. HIF-1 also increases the expression of pyruvate kinase (PK), which converts phosphoenolpyruvate (PEP) to pyruvate; an ATP-generating key step in glycolysis. In its alternatively spliced, embryonic M2 form (PKM2), PK promotes the Warburg effect in cancer cells (fermentation of glucose into lactate despite the presence of adequate pO₂; decreased oxidative phosphorylation), partly by enhancing HIF transcriptional activity through interaction with PHD-3 (PHD-3 hydroxylates PKM2) [96]. PKM2 is selectively expressed in tumor cells, where it promotes tumorigenesis by shifting glucose metabolism towards biomass synthesis in the presence of proliferative signals. Under these conditions an alternative pathway becomes active that produces pyruvate, while decoupling ATP generation from PEP-mediated phosphotransfer [89, 97, 98].

As a result of increased glycolysis, NAD⁺ is consumed and needs to be regenerated to sustain glycolytic flux, which is accomplished by converting pyruvate to lactate via HIF-regulated LDH. To maintain intracellular pH, HIF-1 facilitates the excretion of protons and lactate by increasing the expression of sodium/hydrogen exchanger (NHE)-1 and monocarboxylic acid transporter (MCT)-4 [99]. HIF-1 activation keeps intracellular pH in a slightly alkaline range by up-regulating membrane-bound ectoenzyme carbonic anhydrase (CA) IX, which catalyzes the conversion of CO₂ to bicarbonate, and is highly expressed in RCC and other tumors where it associates with poor prognosis [99].

HIF-1 furthermore affects cellular O₂ consumption by directly regulating mitochondrial metabolism and biogenesis. It suppresses mitochondrial biogenesis through c-MYC inhibition [100], and enhances the efficiency of O₂ utilization by changing the molecular subunit composition of mitochondrial complex IV {cytochrome c oxidase (COX)} [101]. In addition, HIF-1 participates in the control of mitochondrial mass through BCL-2 family member BNIP3, which is involved in the regulation of mitochondrial autophagy [102].

Another metabolic hallmark of VHL-deficient ccRCC is the accumulation of neutral lipids and glycogen, which results in the development of 'clear' cytoplasm, hence the histological term 'clear cell carcinoma'. Lipid accumulation is also present in stromal cells, the neoplastic component of VHL-associated hemangioblastomas [14]. Our laboratory has found that HIF-2 regulates cellular lipid metabolism [103] by suppressing fatty acid β -

oxidation and by increasing lipid storage capacity in VHL-deficient hepatocytes (up-regulation of lipid droplet binding protein ADFP) (Figure 3). In other tissues, HIF promotes lipid synthesis via peroxisome proliferator-activated receptor γ -mediated increase in fatty acid uptake and synthesis [104]. HIF also promotes glycogen synthesis, even under hypoxic conditions. HIF-1 increased phosphoglucomutase 1, glycogen synthase, UTP:glucose-1-phosphate uridylyltransferase 1 and 1,4- α glucan branching enzyme [105].

The pVHL/HIF axis as a therapeutic target

The pVHL/HIF pathway has emerged as major therapeutic target for non-surgical treatment of patients with RCC, and the United States Food and Drug Administration (FDA) has approved several agents for the treatment of RCC. These agents work either directly on HIF-regulated targets and their receptors, such as VEGF, VEGF receptors (VEGFR), platelet-derived growth factor receptor (PDGFR) and other tyrosine kinase receptors, or on mTORC1, which controls cellular growth and increases HIF-1 α translation. A classic example of anti-VEGF therapy is treatment with bevacizumab, an anti-VEGF antibody that has been successfully used for metastatic RCC [106]. Although high dose bevacizumab did not improve overall survival, anti-VEGF therapy did inhibit disease progression. Small molecule tyrosine kinase inhibitors (TKIs), such as sorafenib, sunitinib and pazopanib, have direct antitumor and anti-angiogenic effects. They target the catalytic domains of several tyrosine kinase receptors including VEGFR and PDGFR, and inhibit the RAF/MEK/ERK pathway (Sorafenib). TKIs improved progression-free survival and quality of life, their effects on overall survival, however, were limited [107–109]. Another important therapeutic target is mTOR. Several mTOR inhibitors, Temsirolimus and Everolimus are currently undergoing clinical evaluation for the treatment of advanced RCC [110, 111]. In experimental models, inhibition of mTOR has been shown to reduce tumor growth of VHL-deficient RCC cells, which correlated with decreased HIF activity [74].

Recent studies characterizing the effects of metabolic reprogramming on tumor growth have identified critical control points that regulate how tumors utilize nutrients and O₂ to support proliferation. These control points could become excellent targets for tumor therapy. In RCCs, which are highly glycolytic tumors, pharmacological targeting of metabolic control points could restrict the tumor's ability to utilize nutrients for growth, or could make a tumor more susceptible to chemotherapy. For example, inhibition of PDK-1, which controls the conversion of pyruvate to acetyl CoA, increases mitochondrial O₂ consumption and enhances the effectiveness of the hypoxic cytotoxin tirapazamine [94]. Furthermore, PDK-1 inhibition reduces cell growth of hypoxic cells and is likely to increase susceptibility to apoptosis [95].

While therapeutic efforts in the context of RCC aim at suppressing the effects of HIF activation by either inactivating specific downstream targets or inhibiting HIF- α translation, efforts in other clinical fields are under way to exploit the benefits of HIF activation. Structural analogs of 2-oxoglutarate, which is the substrate for PHD enzymes and FIH, have successfully been used to stimulate endogenous EPO production, and have been shown to protect from ischemic injuries in pre-clinical models [31, 33]. Several compounds, which result in transient inhibition of HIF proteolysis, are currently in clinical trials for the treatment of renal anemia (impaired ability to produce adequate amounts of EPO). Although the biological effects of repeated transient HIF activation are very likely to be different from the effects of constitutive HIF activation (VHL-deficiency), the long term consequences of transient pharmacologic HIF activation with regard to oncogenic potential are unknown, particularly in dialysis patients with acquired cystic disease who are at increased risk for developing RCC [112].

Concluding remarks

Dysregulated O₂-sensing has surfaced as a central and early event in the pathogenesis of RCC. Pre-clinical and clinical investigations into HIF-associated pathogenesis have led to the development of new FDA approved targeted therapies, which have produced promising clinical results. Additional therapeutic targets are likely to emerge as more knowledge is generated about how RCC cells sense and metabolize nutrients. However, from the point of view of basic biology, multiple questions that concern RCC pathogenesis remain unanswered. Despite extensive study of the biological effects of HIF activation in RCC, the sequence and the exact nature of the additional molecular events, which are required for malignant transformation of renal epithelial cells that have undergone HIF activation, remain largely unclear. Answers to these questions are likely to pave the way for the development of new therapies that will ultimately improve the life of patients with RCC.

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Figure 1. HIF has a central role in the pathogenesis of RCC

Mutations in TCA cycle enzymes fumarate hydratase (FH) and succinate dehydrogenase (SDH) result in HIF stabilization, and predispose to certain subtypes of familial RCC. Other RCC-predisposing gene products, c-MET tyrosine kinase receptor, TSC1 and TSC2, and folliculin signal through mTOR, which regulates HIF- α translation. The most common genetic event associated with sporadic RCC is loss of pVHL function. The pathogenesis of VHL-associated ccRCC involves both HIF-dependent and HIF-independent functions of pVHL, as well as other genetic events that ultimately lead to malignant transformation. (A) gross photography of ccRCC; (B) clear cell histology (arrows), magnification $\times 400$. Images were kindly provided by Dr. John Tomaszewski, Department of Pathology, University of Pennsylvania, Philadelphia, PA.

Figure 2. pVHL regulates HIF

Under normoxia, HIF- α is hydroxylated by prolyl-4-hydroxylases and targeted for proteasomal degradation by the pVHL-E3 ubiquitin ligase complex. Binding to hydroxylated HIF- α occurs through pVHL's β -domain, which spans amino acid residues 64–154. The C-terminal α -domain of pVHL (which functions as a substrate recognition component) connects via elongin C to the other components of the E3-ubiquitin ligase complex (Elongin B, Cullin 2 and RBX1). When prolyl-4-hydroxylation is inhibited, e.g. in the absence of molecular oxygen, HIF- α is not degraded and translocates to the nucleus where it heterodimerizes with ARNT. HIF- α /ARNT heterodimers bind to the HIF consensus-binding site, RCGTG, and recruit co-transactivators, followed by transactivation of target genes (e.g. VEGF, PDK-1, EPO). Nitric oxide, reactive oxygen species, TCA cycle metabolites succinate and fumarate, cobalt chloride and iron chelators such as desferrioxamine inhibit HIF prolyl-4-hydroxylases in the presence of oxygen. Aside from targeting HIF- α for proteasomal degradation, pVHL has multiple other functions. These include regulation of primary cilium maintenance, regulation of microtubule stability and interactions with several other signaling pathways. **Abb.:** CoCl₂, cobalt chloride; EPO, erythropoietin; NO, nitric oxide; PDK-1, pyruvate dehydrogenase kinase 1; ROS, reactive oxygen species; ub, ubiquitin; VEGF, vascular endothelial growth factor.

Figure 3. Metabolic reprogramming by HIF

Overview of HIF-regulated enzymatic steps in glycolysis and mitochondrial metabolism. Green arrows depict promoting effects; red arrows indicate inhibition. Green letters highlight HIF-regulated transcriptional targets (increased expression). HIF-1 activation increases the expression of several glycolytic enzymes (see main text). Increased glycolytic flux results in increased production of lactate, which is excreted via MCT-4. HIF-controlled CA IX and NHE-1 maintain intracellular pH. PDK-1 inhibits the enzymatic activity of pyruvate dehydrogenase, which converts pyruvate to acetyl-CoA. As a result mitochondrial O₂ consumption is diminished. The mitochondrial electron transport chain is depicted by rectangles. SDH is part of ETC complex II (yellow rectangle) and the TCA cycle. Furthermore, HIF regulates the subunit composition of mitochondrial complex IV (blue rectangle). HIF-1 increases COX4-2 expression directly and diminishes COX4-1 expression by enhancing COX4-1 degradation through up-regulation of LON. In contrast to HIF-1, HIF-2 is responsible for neutral fat accumulation. HIF-2 increases the expression of ADFP (direct transcriptional target), inhibits fatty acid synthesis and β-oxidation in VHL-deficient hepatocytes. **Abb.:** ADFP, adipose differentiation-related protein; CA IX, carbonic anhydrase 9; COX4, cytochrome c oxidase subunit 4; ETC, electron transport chain; GLUT-1, glucose transporter 1; HK, hexokinase; LON; mitochondrial LON protease;

MCT-4, monocarboxylic acid transporter 4; NHE-1; Na⁺/H⁺ exchanger 1; PEP, phosphoenol pyruvate; PKM2, pyruvate kinase isoform M2.