

Hypoxia, angiogenesis, and metabolism in the hereditary kidney cancers

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The field of hereditary kidney cancer has begun to mature following the identification of several germline syndromes that define genetic and molecular features of this cancer. Molecular defects within these hereditary syndromes demonstrate consistent deficits in angiogenesis and metabolic signaling, largely driven by altered hypoxia signaling. The classical mutation, loss of function of the von Hippel-Lindau (VHL) tumor suppressor, provides a human pathogenesis model for critical aspects of pseudohypoxia. These features are mimicked in a less common hereditary renal tumor syndrome, known as hereditary leiomyomatosis and renal cell carcinoma. Here, we review renal tumor angiogenesis and metabolism from a HIF-centric perspective, considering alterations in the hypoxic landscape, and molecular deviations resulting from high levels of HIF family members. Mutations underlying HIF deregulation drive multifactorial aberrations in angiogenic signals and metabolism. The mechanisms by which these defects drive tumor growth are still emerging. However, the distinctive patterns of angiogenesis and glycolysis-/glutamine-dependent bioenergetics provide insight into the cellular environment of these cancers. The result is a scenario permissive for aggressive tumorigenesis especially within the proximal renal tubule. These features of tumorigenesis have been highly actionable in kidney cancer treatments, and will likely continue as central tenets of kidney cancer therapeutics.

Introduction

Hereditary cancers in the kidney have provided a wealth of material to advance our understanding of cellular physiology. Most notably in the context of the mutation in the von Hippel-Lindau (VHL) gene, carriers display an autosomal dominant risk for clear cell-type renal cell carcinoma (ccRCC) and other lesions throughout the body (1). This syndrome is an exceptionally valuable teaching tool in the medical school setting, illustrating concepts of hereditary cancer risk, loss of heterozygosity, sensor-driven signaling, genetic defects altering protein level regulation (ubiquitin/proteasome degradation) as opposed to transcriptional regulation (transcription factors), tumor cellular metabolism, and growth factor-driven angiogenesis, among other topics. Studies in the biology of VHL disease and other kidney cancer syndromes led to key discoveries that cemented angiogenesis and metabolism as hallmarks of cancer. This Review will consolidate our knowledge around angiogenesis and metabolism, as learned from the vantage point of hereditary kidney cancer (Figure 1).

VHL disease: an illustration of angiogenesis-fueled hereditary disease

The VHL gene was discovered in 1993 in the pursuit of a familial autosomal dominant syndrome of numerous highly vascular tumors (2). Originally described as a syndrome, also known as familial cerebellar retinal angiomatosis, it was recognized in the

early 1900s by German ophthalmologist Eugen von Hippel, who described angiomas in the eye in 1904, and Swedish pathologist Arvid Lindau described the angiomas of the cerebellum and spine in 1927 (3, 4). The term von Hippel-Lindau disease was first used in 1936; however, its use became common only in the 1970s. VHL disease's link to ccRCC was discovered much later, but as this tumor is also characterized by a vascular-rich malignancy, the association was natural.

VHL disease has a prevalence of 2–3 per 100,000 and an estimated incidence around 1 of 45,000 live births (5). Penetrance is 90% by age 65, and the manifestations emerge over the lifetime of affected individuals.

The VHL gene is located on chromosome 3p25.1. The VHL protein (pVHL) is composed of at least two isoforms, both of which convey activity as the substrate-binding component of an E3 ubiquitin ligase complex. VHL is the centerpiece of a finely tuned rheostat system that regulates the response to low oxygen levels. Several targets of pVHL-mediated proteasomal degradation have been reported, but the canonical substrates include the hypoxia-inducible factors (HIF-1 α and HIF-2 α). These transcription factors interact with pVHL via their oxygen-dependent degradation domains (ODDs) containing proline residue targets that undergo hydroxylation by a family of iron- and oxygen-dependent prolylhydroxylation (PHD) enzymes. Thus, in the presence of physiological oxygen levels, PHD enzymes place a prolylhydroxylation mark on the HIF ODD, rendering HIF proteins susceptible to pVHL-mediated ubiquitylation and proteolytic degradation via the proteasome. Levels are kept low until there is a deficit in oxygen supply, at which point unhydroxylated HIF proteins are free to accumulate, heterodimerize with their obligate partner protein (HIF-1 β , also known as the aryl hydrocar-

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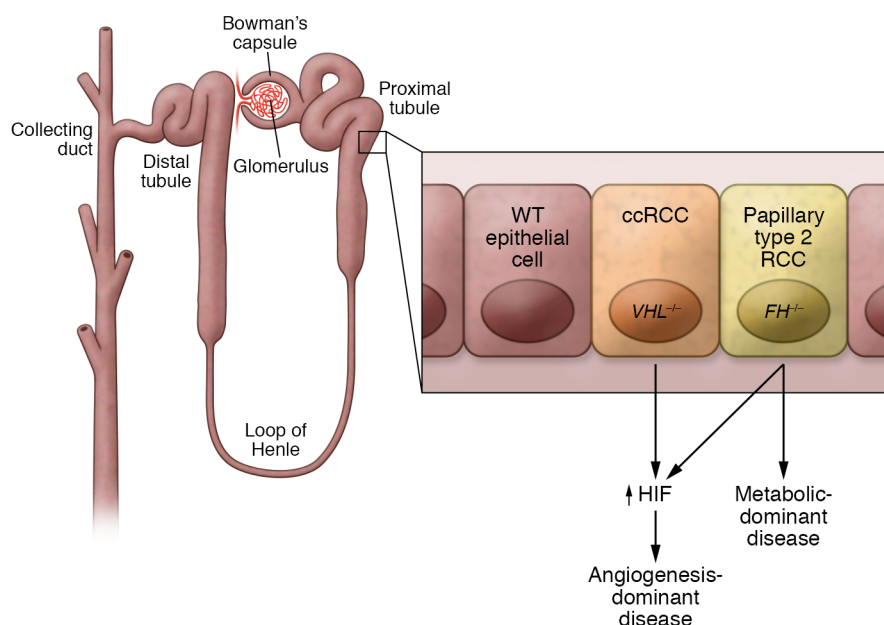


Figure 1. Common themes in hereditary kidney cancer syndromes. The two dominant forms of hereditary (and sporadic) RCC derive from cells in the proximal tubule. In spite of their common or similar origin, these tumor types have distinct genetics and biological characteristics. Although both VHL mutation, which is associated with clear cell type RCC, and FH mutation, which is associated with papillary-type 2 RCC, can deregulate HIF expression, these factors drive a differing balance of angiogenic and metabolic features, contributing to the overall pattern of the distinct diseases.

bon receptor nuclear translocator [ARNT]), and execute their function as potent transcription factors (Figure 2).

In the absence of pVHL, HIFs promote a unique angiogenic state of continuous mitogenic signaling. Thus, numerous investigations have detailed the effect of VEGF signals in this context. However, in the context of VHL disease, as well as in ccRCCs in general, a variety of *VHL* mutations, of varying severity, are observed. Full genomic deletions are common, along with frameshift and truncating mutations, as are point mutations, which result in protein instability and mutations that retain partial functionality of the protein. Moreover, this disease gives rise to significant genotype-phenotype correlation, and the spectrum of proangiogenic effects varies according to the class of mutations that are observed.

VHL disease is subdivided into type 1 and type 2. Type 1 conveys high risk for ccRCC, and is typically caused by complete loss of the protein. Type 2 is associated with missense mutations, along with risk for the syndrome of pheochromocytoma or paraganglioma. Type 2 is further subclassified based on risk of developing hemangioblastomas (type 2A with lower risk, type 2B with the highest risk) (6). Type 2C, typified by the L188V mutation, conveys risk for pheochromocytoma alone, and preserves HIF regulatory function (7). Finally, homozygosity for a rare mild mutation at the extreme C terminus, R200W, causes a familial autosomal recessive syndrome of erythrocytosis and polycythemia via a more subtle, and context-dependent, effect on HIF-2 α deregulation (8–10).

The common theme in differentiating these mutations is the extent to which pVHL interacts with and differentially regulates the canonical targets HIF-1 α and HIF-2 α (11). The differential impact of stable expression of one versus the other of these factors has direct implications for angiogenic signaling and will be discussed in detail later in this Review.

HLRCC: an illustration of metabolically driven hereditary kidney cancer

A second hereditary kidney cancer syndrome, called hereditary leiomyomatosis and RCC (HLRCC), provides additional insights

into the core mechanisms of tumor cell fitness (12). HLRCC is another classical tumor suppressor autosomal dominant disease that conveys risk for leiomyoma (with leiomyomas in the uterus and skin predominating) and papillary-type 2 RCC. This syndrome is caused by germline mutations in a core Krebs cycle enzyme, fumarate hydratase (FH) (13). FH loss uncouples the Krebs cycle, driving up fumarate levels, and impairing cellular oxygenation as a result of the lack of reducing substrate (NADH) to drive electron transport (Figure 2).

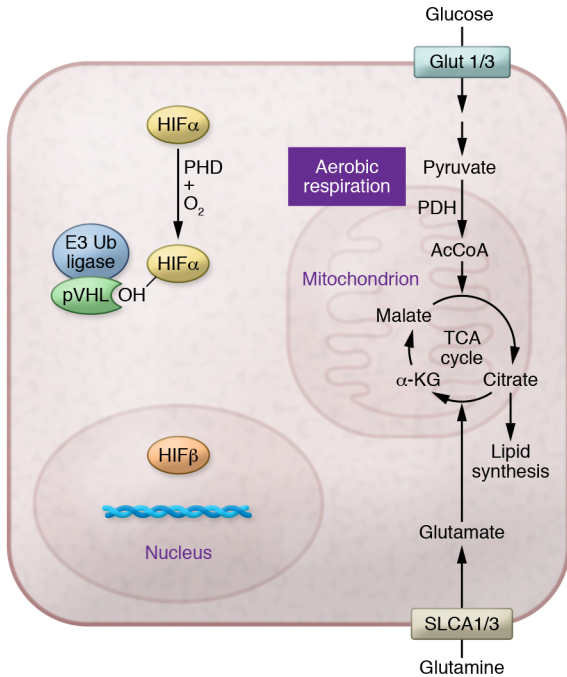
How the FH mutation contributes to the development of a highly invasive and lethal kidney cancer remains an issue of active investigation. FH-mutant tumor cells are highly dependent on glycolysis and conduct reductive carboxylation, essentially reversing the Krebs cycle as a result of substrate availability. Constitutive HIF stabilization further contributes to metabolic aberrance in this cancer (14, 15). In FH-mutant tumor cells, accumulated fumarate mimics α -ketoglutarate to directly inhibit PHD proteins. The result is lack of hydroxylation and pseudohypoxic stabilization of HIF factors in ccRCC (16).

A handful of other familial syndromes of kidney cancer risk (17) lend additional insight into the essential mechanisms of renal tumorigenesis. Birt-Hogg-Dubé syndrome conveys risk for a variety of RCC subtypes due to mutations in the folliculin (*FLCN*) gene. Tuberous sclerosis causes angiomyolipomas and risk for ccRCC due to mutations in *TSC1* or *TSC2* (tuberous sclerosis complex 1 and 2) that lead to activation of mTOR as well as HIF upregulation via enhanced cap-dependent translation; and hereditary papillary RCC, caused by activating mutations in *cMET*. Each of these mutations directly or indirectly impacts HIF signaling, tumor angiogenesis, and metabolism.

HIF/hypoxia, a central mediator of renal tumor risk

HIF α /HIF β complex, hypoxia, and transcription activity. The capacity to detect and adapt to changes in oxygen is critical for cellular and whole-organism homeostasis, representing a critical

A Normoxic cellular metabolism



B VHL-deficient RCC metabolic reprogramming by HIF

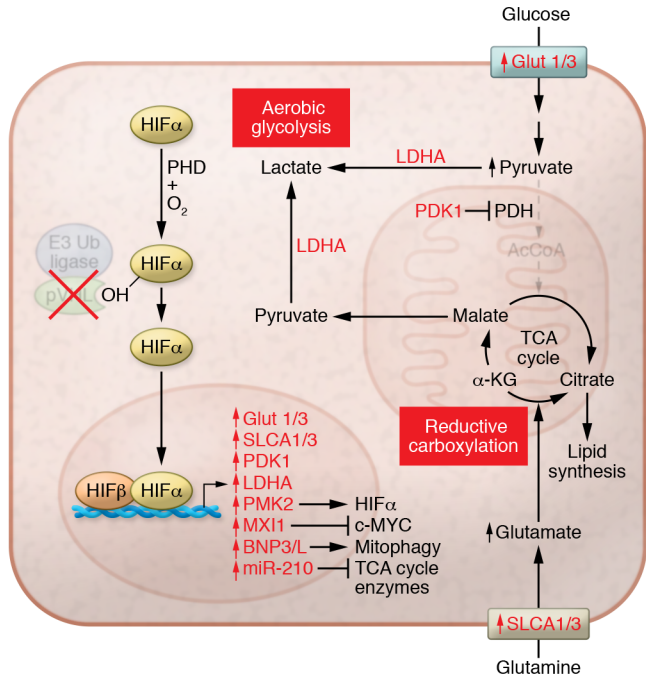


Figure 2. HIF-mediated metabolic reprogramming in VHL-deficient RCC. Germline mutations that render the tumor suppressor gene *VHL* defective, as observed in a majority of clear cell renal carcinoma cells (ccRCC), interfere with pVHL-mediated proteolysis of HIF α (compare a classic model of cellular metabolism in **A**, with pseudohypoxic HIF-driven RCC metabolic reprogramming in **B**). Stabilized HIF α translocates to the nucleus, where it dimerizes with HIF β and directly upregulates transcription of genes related to cellular metabolism, among hundreds of others. HIF reprograms metabolism away from aerobic respiration and toward aerobic glycolysis by increasing conversion from pyruvate to lactate (via upregulation of LDHA) and by blocking pyruvate conversion to acetyl-CoA by PDH (via upregulation of PDK1). HIF increases metabolic nutrients by upregulating transporters for both glucose (GLUT1 and GLUT3) and glutamine (SLCA1 and SLCA3), thereby increasing rates of glycolytic and reductive carboxylation pathways, respectively. In addition, HIF mediates a reduction in aerobic respiration by upregulating BNIP3 and BNIP3L, which leads to selective mitochondrial degradation. HIF interferes with TCA cycle enzymes via miR-210, which disrupts formation of Fe-S clusters necessary for catalysis. Upregulation of the transcription suppressor MXI1 represses c-MYC expression that greatly facilitates the metabolic shift in cancer cells. HIF amplifies its own transcriptional activity by upregulating the HIF α cofactor PKM2. Ub, ubiquitin. α -KG, α -ketoglutarate.

evolutionary adaptation of multicellular organisms and enabling survival over time. Nearly every mammalian cell (18) responds to reduced oxygen availability through activation of the transcription factor HIF (19–22).

When conditions allow the two isoforms of HIF α , HIF-1 α and HIF-2 α , to be stabilized, they are subsequently translocated into the nucleus via binding with HIF-1 β (23). There, the HIF α/β dimer binds to hypoxia response elements (HREs) located often in the proximal promoters of target genes, promoting their transcription. Interplay with other DNA-binding proteins enables cooperative binding or coactivation of HIF, fine-tuning the activation of HIF targets (20, 24). While there is some redundancy between HIF-1 and HIF-2 targets, inactivation of each leads to unique phenotypes, perhaps due to their tissue-specific and temporally specific expression patterns (25–27).

In the presence of hypoxia, HIF activation reprograms cellular oxidative metabolic mechanisms, representing an elegant bioenergetic adaptation enabling cells to mitigate toxic reactive oxygen species (ROS) and to preserve macromolecular synthesis in response to oxygen availability. The reprogramming of numerous and varied cellular systems by HIF in tumorigenesis, including stem cell maintenance, growth factor signaling, epithe-

lial-mesenchymal transition, invasion, metastasis, angiogenesis, and metabolism (28–31), underscores the consequential role of HIF in cancer progression.

HIF-1 versus HIF-2. Both HIF-1 α and HIF-2 α appear to be involved in ccRCC initiation (32, 33). However, it is thought that HIF-1 α functions as a tumor suppressor in ccRCC by attenuating VHL-deficient tumor cell proliferation (34) and is not active in some ccRCCs (35, 36). Deletions of chromosome 14q, which harbors the *HIF-1A* locus, occur in ccRCC and indicate poorer outcomes (37–39). Conversely, HIF-2 α consistently functions as an oncoprotein in ccRCC (34, 36, 40). In the VHL-deficient setting, HIF-2 α upregulates targets involved in angiogenesis (41–44), oxidative stress resistance (45), mitochondrial biogenesis (34, 46–48), metastasis (49, 50), and autonomous proliferation and cell cycle (36, 43, 51, 52). In addition, HIF-2 α , but not HIF-1 α , can override pVHL’s suppressive function (34, 40, 53). Consequently, ccRCCs that express only HIF-2 α are characterized by increased cell proliferation and adverse prognosis (36, 45, 54, 55). Elimination of HIF-2 α in vivo attenuates tumorigenesis in VHL-deficient RCC cells (56, 57). These and other differential impacts of HIF-1 α versus HIF-2 α (58) present exploitable and attractive mechanisms for targeted therapies in ccRCC.

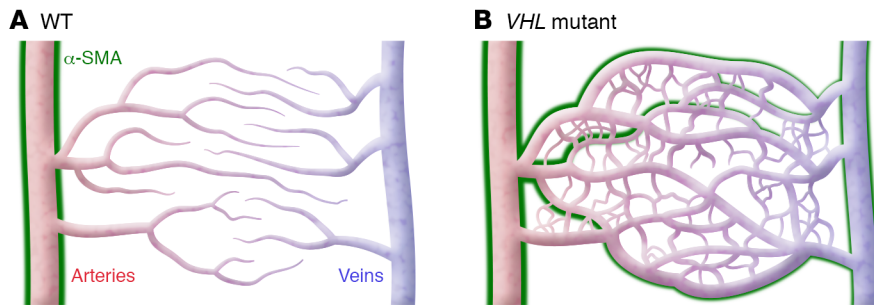


Figure 3. Vascular dysmorphogenesis during VHL mutations. Inducing *VHL* mutations experimentally (compare WT conditions in **A** with *VHL* mutant condition in **B**) leads to vascular abnormalities characterized by an ectopic expression of smooth muscle α -actin (α -SMA; green) by vascular pericytes and vascular patterning defects, including elevated vessel density and the development of arteriovenous shunts spanning major arteries/arterioles (light red) and venules/veins (light blue).

Angiogenesis, a theme in hereditary kidney cancer risk

Misregulated vascular growth and remodeling contribute to the onset and progression of numerous tumor types (59), and these processes are particularly relevant to hereditary kidney cancers. Blood vessel development matches metabolic activity and tissue oxygenation under normal conditions (60). However, disrupted metabolism and oxygen sensing mechanisms in inherited kidney cancers induce structural changes of the blood vasculature, seen prominently in vascular remodeling downstream of HIF misregulation (61, 62), often via *VHL* mutations (63–65). Tumor angiogenesis, or growth of new vessels from existing blood vasculature, is driven primarily by aberrant increases in VEGF-A, which is often induced by tumor hypoxia as the lesion expands, but also results from primary HIF pathway defects (64). Perturbations within other phases of vascular growth (66, 67) also contribute to and accelerate tumor vascularization. For instance, PDGF-BB signaling, an essential pathway for vessel maturation through mural cell recruitment (68–71), can be disrupted directly and indirectly by HIF-VEGF misregulation (72), especially in the tumor context (73–77). Notch signaling has also been implicated in promoting tumor vascularization (78–82), as Notch receptors and ligands regulate not only endothelial phenotypic heterogeneity during sprouting angiogenesis (72, 83, 84), but also arterial-venous specification (85–87) and vessel maturation via mural cell investment (88–90). In addition to crosstalk within these signaling networks, molecular cues from the angiopoietin (ANGPT)/Tie pathway also coordinate the balance between (a) vascular plasticity and endothelial cell sprouting, primarily via ANGPT2-induced destabilization (91), and (b) vessel maturation via long-term investment of pericytes and vascular smooth muscle cells, which occurs downstream of ANGPT1-Tie2 interactions (92). In the context of renal cancer vascularization, these molecular pathways, among others, contribute to a complex pattern of angiogenesis and neovessel formation, as well as to the misregulation of vessel stabilization and maturation (93). They may, therefore, offer unique targets for modulating not only initial tumor vascularization, but also additional vessel remodeling processes that likely exacerbate tumor progression and undermine effective chemotherapeutic delivery.

Angiogenesis in VHL disease – beyond the kidney

Aberrant vascular remodeling often occurs in hereditary kidney cancer diseases – VHL disease being a notable example. High metabolic demand and oxygen consumption within the kidney

compound disease-related defects in the mechanisms regulating these activities, thereby fueling the excessive proangiogenic signaling described in the previous section. These defects can lead to disease manifestations in tissues beyond the kidney, where abnormal vascular growth and remodeling contribute to additional pathologies. For instance, brain, spinal cord, and eye/retina hemangioblastoma formation is commonplace in VHL disease (94, 95), owing in part to disruption of the VEGF-A (96, 97) and Notch pathways (98), among others. Dysfunction in HIF signaling is also likely involved in the aberrant vessel remodeling found in nonkidney tissues, as recent studies have implicated this pathway, and downstream mediators such as ANGPT-like 4 (ANGPTL4), in angiogenesis-related conditions including pterygia (99), uveal melanoma (100), and proliferative retinopathies (101–103).

Increased insight into how angiogenesis defects lead to clinical manifestations of ocular VHL disease, such as retinal capillary hemangioblastoma (RCH) formation, will advance future therapies as well as enhance the diagnostic strategies of ophthalmological examination of VHL patients (104–106). To better understand retinal vascular malformations in the *VHL* mutation scenario, we recently examined the retinal vasculature using inducible mouse models of type 1 (null) and type 2B (murine G518A representing human R167Q) *Vhl* mutations (6, 11, 107). Retinal vessels of type 2B *Vhl*-mutant animals displayed hallmarks of an accelerated progression toward an arterial phenotype, including ectopic expression of vascular smooth muscle contractility proteins in microvascular pericytes (Figure 3 and ref. 98). We further found that both types 1 and 2B genetic mutations resulted in abnormal angiogenic remodeling and changes in stage-specific vascular density (Figure 3 and ref. 98). These observations were consistent with a zebrafish model of VHL-associated retinopathy in which retinal angiogenesis and vessel leakage contributed to macular edema and retinal detachment (108). Blocking VEGF signaling in this type 1 *Vhl*^{-/-} model improved retinopathy outcomes (108), though recent studies have cautioned against sustained anti-VEGF interventions in the eye, as they may have deleterious effects on retinal neurons (109–111). Current treatment of ocular VHL disease includes systemic or intravitreal administration of anti-VEGF agents, laser photocoagulation, and cryotherapy (112), though clinical management remains a challenge because of the likelihood of new RCH formation and the frequent presence of multiple lesions in both eyes.

Aberrant blood vessel formation also gives rise to hemangioblastomas in the cerebellum and spinal cord of VHL patients (94, 113, 114). Similarly to the kidney, these tissues have high metabolic

demands and minimal to no energy reserves, which exacerbate genetic defects in mechanisms regulating metabolism and oxygen sensing (114). While VHL-mediated kidney cancer involves vascular remodeling via angiogenesis, hemangioblastoma formation in neurological tissues of VHL patients may also involve vasculogenic processes (113, 115, 116). The precise cellular contribution of vascular cells versus tumor “stromal” cells to the dense vascularity of hemangioblastomas remains an open question (113); it is clear, however, that excessive proangiogenic factors such as VEGF-A, PDGF-BB, and EGF drive lesion growth (117, 118). For these reasons, antiangiogenesis strategies initially developed to treat kidney cancers, and specifically VHL-associated ccRCC, are being adapted to manage CNS hemangioblastomas untreatable by surgical resection or radiation therapy (117, 119, 120).

Antiangiogenic targeting in RCC

Altered hypoxia signaling and metabolism in renal cell carcinoma, as seen in VHL disease (121), drives angiogenic pathway activation, leading to the rationale for targeting signals involved in vascular remodeling (122, 123). Development of anti-VEGF therapies in particular has focused on dampening VEGF signaling by reducing ligand levels (e.g., the VEGF-A-targeting antibody bevacizumab) or interfering with tyrosine kinase activity and receptor phosphorylation (e.g., axitinib, pazopanib, sorafenib, sunitinib). These agents transformed the care of patients with RCC (122). Increasing appreciation for proangiogenesis resistance mechanisms (124) has inspired development of agents targeting additional growth factor pathways such as FGF (lenvatinib, targeting FGFR) and HGF (cabozantinib, targeting the HGF receptor cMET). Clinical trials involving anti-VEGF therapy for RCC patients (125, 126) highlight the need for increased insight into more effective uses of antiangiogenic agents, potential combinatorial approaches such as with immunotherapy treatment (127, 128), and additional molecular targets intersecting with VEGF signaling.

Because HIF signals provide critical regulation of VEGF-A activity, this pathway has gained significant attention in the development of antiangiogenesis therapies. For example, acriflavin, which blocks HIF-1 dimerization, has shown promise in reducing tumor growth and associated angiogenesis in preclinical models (129, 130). Multimodal therapy involving HIF-1 α inhibition alongside VEGF-A inhibition and hypoxia-activated chemotherapy inhibits angiogenesis and cancer stem cell-like proliferation/survival in sarcomas (131), warranting validation of this approach for treating RCC. HIF-2 antagonists (such as PT2399 and PT2385) are also in development and show early indications of efficacy in treating a subset of ccRCCs (41, 132, 133), though divergent responses to HIF-2 inhibition underscore the importance of careful inclusion of key biomarkers in clinical trial design (134).

Hypoxia and metabolism in promoting kidney cancer risk

Glycolysis and glutaminolysis. Uncontrolled proliferation of cancer cells requires increased synthesis of cellular components such as amino acids, lipids, and nucleotides, to meet basic tumor demands. Under normal oxygen conditions, energy is generated by the complete oxidation of glucose via aerobic respiration. However, independent of oxygen availability, cancer cells transition

to aerobic glycolysis that promotes anabolic metabolic flux (135). This metabolic reprogramming, referred to as the Warburg effect in cancer cells (136), is mediated by HIF-1 (52, 137–140). To offset the energetic inefficiency of glycolytic metabolism, HIF-1 activates expression of the glucose transporters GLUT1 and GLUT3 to increase glucose uptake for glycolysis (141, 142). In addition, HIF-1 reprograms metabolism by inducing expression of glycolytic enzymes (142–148). In particular, HIF-1 inhibits conversion of pyruvate to acetyl-CoA (AcCoA) by pyruvate dehydrogenase (PDH) and subsequent entry into the TCA cycle, by regulating (a) *PDK1*, encoding pyruvate dehydrogenase kinase 1 (149, 150), which inactivates PDH; and (b) *LDHA*, encoding lactate dehydrogenase A (142, 151), which converts pyruvate to lactate in glycolytic metabolism. In so doing, HIF-1 shuttles glucose away from respiration and into glycolysis. HIF-1 also activates mitochondrial-selective autophagy via regulation expression of BCL-2 family member BNIP3 and its ligand, BNIP3L, thereby preventing glucose and fatty acid oxidative metabolism (152, 153). Additionally, HIF-1 interferes with components of the TCA cycle and electron transport chain via activation of microRNA-210 (miR-210) (154–156). However, despite these diverse strategies of HIF-1 to reprogram metabolism, oxidative respiration is not completely abolished. Consequently, by activating the Lon protease (*LON*) gene, HIF-1 improves efficiency of electron transport (157). However, overall reduction in electron transport efficiency in hypoxic conditions leads to increased ROS (158). The switch from oxidative to glycolytic metabolism pertains to ATP maintenance as well as toxic oxidant accumulation (139).

HIF's metabolic reprogramming of cancer cells is amplified by the glycolytic enzyme pyruvate kinase M2 (PKM2) in a positive-feedback mechanism. As an alternative splice product encoded by the *PKM2* gene (159), PKM2 is expressed in the embryo and in cancer cells. In catalyzing the conversion of phosphoenolpyruvate to pyruvate, PKM2 is an important determinant in the glycolytic pathway. PKM2 contributes to enhanced lactate production seen in some cancer cells following hydroxylation by PHD3, potentiating PKM2 function as a HIF-1 coactivator (160). In turn, this coactivation leads to transactivation of HIF-1 target genes, which includes those encoding both PHD3 and PKM2. The HRE located in the proximal *PKM2* promoter is recognized and activated by HIF-1 α and HIF-1 β , but not HIF-2 α (160). Therefore, by elevating PKM2 and PHD3 expression, HIF-1 may boost its own activity and enhance the Warburg effect observed in cancer cells (160).

In addition to glucose, glutamine is a key energy-producing nutrient that supports proliferating cells (Figure 2). Reductive glutamine metabolism provides vital metabolic intermediates for macromolecule synthesis. In hypoxic or highly proliferating cells, such as cancer cells, glutamine is not fully oxidized, but is rather used to generate citrate through reductive carboxylation (RC) of α -ketoglutarate to provide intermediates (e.g., AcCoA) for lipid synthesis, which is otherwise primarily fueled by glucose-derived pyruvate (161–165). *VHL*-deficient RCC cells, which show constitutive activation of HIF-1 α and/or HIF-2 α (166), synthesize lipids via RC-derived AcCoA rather than through glycolysis. Glucose-derived lipid synthesis is restored in this setting following introduction of wild-type *VHL* (163), demonstrating a HIF-mediated metabolic shift to RC in the *VHL*-deficient cells.

A prominent mechanism by which HIFs execute this metabolic shift and thereby abrogate cellular respiration is through the oncogenic transcription factor c-MYC, known to induce proliferation. In contrast to HIF-2 α (61), HIF-1 α inhibits c-MYC activity both through transcriptional repression, by activating the GTP-binding protein MXI1, and through targeted proteolysis in VHL-deficient RCC. Consequently, the metabolic transcriptional profile in VHL-deficient ccRCC is altered with HIF-1-mediated loss of c-MYC, enhanced by the concomitant loss of the c-MYC-dependent transcriptional coactivator PGC-1 β (52).

In conjunction with reductive metabolism of glutamine, HIF also influences glutamine signaling (167). The function of glutamate receptors is well documented in various cancer types (168), and HIF enhances glutamine signaling to drive tumor progression. Specifically, HIF triggers expression of AMPA-type glutamate receptors and membrane glutamate transporters that activate SRC family kinases and related signaling pathways. As a result, proliferation, survival, migration, and invasion are enhanced in ccRCCs and VHL-null cells (167).

Alternative regulatory metabolic features. As an example of the broad-reaching impact of metabolic derangement related to HIF biology, O-GlcNAcylation, the posttranslational process by which O-linked β -N-acetylglucosamine (O-GlcNAc) is added to intracellular proteins, impacts the hydroxylation of HIF-1 in cancer cells. O-GlcNAc modifies intracellular proteins directly or indirectly as a response to changes in nutrient levels or stress (169). Elevated levels of O-GlcNAcylation have been reported in cancers (170) and are indicated in the reprogramming of cancer cell metabolism (171). HIF-1 α hydroxylation by PHD, interaction with pVHL, and proteasomal degradation are regulated by O-GlcNAcylation, though not via direct O-GlcNAc modification. Further, O-GlcNAcylation-mediated changes in metabolic flux required HIF-1 α hydroxylation in vitro (171).

HIF activities extend beyond angiogenesis in influencing the microenvironment. HIF expression in immune cells induces various aspects of host innate and adaptive immune function in response to hypoxia, triggering tissue damage and immune cell dysfunction (172). In the microenvironment of ccRCC, as a brief example, CD8⁺ T cells harbor distinct metabolic defects that restrict their ability to activate in response to conventional stimuli (173). Much more work is needed to fully understand the impact of the unique metabolic features of kidney tumors to alter the spectrum of tumor promotion.

Imaging angiogenic and metabolomic defects in RCC risk

Recent advances in medical imaging technology have expanded the clinical armamentarium for assessing and managing kidney cancer risk, specifically through monitoring of tumor-associated vascular remodeling and metabolic defects. Dynamic contrast-enhanced MRI (DCE-MRI) is one such modality that facilitates noninvasive evaluation of RCC blood perfusion and microvessel leakage, correlative indicators of increased angiogenesis (174–176). Perfusion CT offers additional insight into tumor vascularity and blood flow (177, 178), with dynamic enhanced CT of RCC tumors capturing spatial heterogeneities and “hot spots” of increased microvascular density (179). PET, in conjunction with

markers such as ¹⁵O-labeled water (H₂¹⁵O), can also be applied to measure tumor blood flow (180, 181), but challenges remain in applying this modality for longitudinal assessment of RCC (180). Advances in ultrasound imaging have further extended the clinical utility of this imaging technique, as Doppler perfusion imaging and 3D scanning methods provide real-time measures of tumor morphology and vascularity (180, 181). Tumor vascularization imaging by ultrasound, along with MRI and PET, has recently benefited from the development of molecular imaging strategies whereby proteins involved in angiogenesis are labeled by contrast agents (182–184). Sprouting endothelial cells express high levels of VEGFR-2 to mediate VEGF signaling and the integrin $\alpha_v\beta_3$, which facilitates migration along surrounding extracellular matrix. These proangiogenic mediators, among others, have received significant attention in the development of molecular imaging techniques (185–188). Continued innovation in developing cancer imaging modalities and contrast enhancement will expand our understanding of the biology underlying RCC angiogenesis and enhance clinical care in monitoring treatment efficacy and disease progression using vascular biomarkers.

Metabolomic defects in RCC offer another disease feature that can be assessed noninvasively via the aforementioned imaging modalities (189). In particular, elevated glucose uptake owing to increased metabolic activity in RCC can be monitored using ¹⁸F-labeled fluorodeoxyglucose (FDG) in conjunction with PET/CT, particularly in detecting metastatic lesions (190–193). Variability in tumor FDG uptake, however, remains a key limitation in assessing primary RCCs (194) and can also hamper comprehensive evaluation of secondary RCC metastases, which may exhibit differential expression of glucose transporters and therefore wide-ranging FDG uptake capacities (195). For these reasons, complementary approaches are being developed to harness RCC metabolic defects for diagnostic imaging. Carbon-11 (¹¹C)-acetate, for instance, can be rapidly taken up by tumor cells and converted to AcCoA, which contributes to synthesis of cell membrane fatty acids, a process that is accelerated during tumor cell proliferation (196). Coupled with PET imaging, this tracer has shown promise in predicting RCC response to the tyrosine kinase inhibitor sunitinib (196, 197). Membrane lipid synthesis also involves the generation of phosphatidylcholine following choline consumption by tumor cells (198), thus providing a rationale for developing ¹¹C-choline PET/CT as a complementary approach to evaluating primary RCC and associated metastatic disease (199). Accelerated tumor cell proliferation can also be exploited for diagnostic imaging by administering ¹⁸F-fluorothymidine and using PET to detect the accumulation of this tracer within tumor cells, as this analog of thymidine cannot incorporate into tumor cell DNA but remains trapped intracellularly following phosphorylation (200). Further development of these metabolomic imaging markers, alongside vascular-based approaches, continues to expand the range of diagnostic tools available for managing and treating RCC risk and progression.

Conclusion

The family of hereditary RCCs have contributed enormous insight into the mechanisms of angiogenesis and associated changes in metabolism that not only facilitate tumor growth, but

act as drivers in the process of tumorigenesis. These core features of the RCC family of tumors have led to new insights in cancer biology and normal cellular physiology. Importantly, they also have satisfied the urgent need to create avenues for therapeutic intervention, and clinical tools such as imaging diagnostics, to enhance the care of patients with cancer. The future holds novel ways to integrate these tools into evolving treatment paradigms, such as immunotherapy, and to benefit the patients with familial syndromes themselves, through early detection, prevention, and therapeutic intervention.

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