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# Metabolic alterations in renal cell carcinoma

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ABSTRACT

Renal cell carcinoma (RCC) is a metabolic disease, being characterized by the dysregulation of metabolic pathways involved in oxygen sensing (VHL/HIF pathway alterations and the subsequent up-regulation of HIF-responsive genes such as VEGF, PDGF, EGF, and glucose transporters GLUT1 and GLUT4, which justify the RCC reliance on aerobic glycolysis), energy sensing (fumarate hydratase-deficient, succinate dehydrogenase-deficient RCC, mutations of HGF/MET pathway resulting in the metabolic Warburg shift marked by RCC increased dependence on aerobic glycolysis and the pentose phosphate shunt, augmented lipogenesis, and reduced AMPK and Krebs cycle activity) and/or nutrient sensing cascade (deregulation of AMPK–TSC1/2–mTOR and PI3K–Akt–mTOR pathways). We analyzed the key metabolic abnormalities underlying RCC carcinogenesis, highlighting those altered pathways that may represent potential targets for the development of more effective therapeutic strategies.



## Introduction

Kidney cancer is a metabolic disease. Most of the genes typically mutated in renal cell carcinoma (VHL, MET, FLCN, FH, SDH, TSC1, and TSC2) have a fundamental role in the regulation of cellular metabolic processes, suggesting a dysregulation of metabolic pathways involved in oxygen, energy and/or nutrient sensing as a key feature of RCC carcinogenesis (Table 1) [1]. A deep understanding of the crucial metabolic abnormalities underlying RCC development should provide a unique opportunity for the development of novel more effective therapeutic strategies.

### *VHL/HIF pathway – oxygen sensing*

Clear cell renal cell carcinoma (ccRCC), the most common adult renal cancer histotype (accounting for 75% of all RCCs), is histologically characterized by a high tumor cell lipid content and a richly vascularized tumor stroma. Molecularly, ccRCC peculiar features concern genes involved in the regulation of angiogenesis. VHL tumor suppressor gene, which is located on the short arm of chromosome 3 at cytoband 3p25–26, can harbor both germline and sporadic mutations. Variations lying in VHL gene and that are able to inactivate the related protein, mark the inherited multisystem cancer von Hippel–Lindau disease [2,3] and also occur in the vast majority of sporadic ccRCC (approximately 90%) [4–6]. This high mutational rate of VHL leads to consider VHL-alterations as an early event in ccRCC carcinogenesis.

The VHL gene encodes two different isoforms of VHL protein (pVHL): a 213-amino-acid, 30 kDa form (pVHL30), and a 160-amino-acid, 19 kDa form (pVHL19), the latter predominates in many tissues. The two pVHL isoforms share equivalent effects and have tumor suppressor activity *in vivo* [7]. pVHL is a multifunctional adaptor protein that forms a ternary complex with the transcription elongation factors elongin C (TCEB1) and elongin B (TCEB2) [8], and interacts with several enzymatic protein partners to mediate ubiquitin-dependent hypoxia inducible factor 1 alpha (HIF1α) and HIF2α degradation in normoxic conditions [9].

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**Table 1**
Deregulated genes and affected pathway.

| Impaired/deregulated genes | Downstream genes | Affected functions |
|---|---|---|
| VHL | HIF1, HIF2, VEGF, PDGF, EGF, GLUT1, c-Myc, CCND2, E2F1, p21, p27 | Angiogenesis, ECM remodeling, genomic integrity, glycolytic enzyme expression, anabolic biosynthesis, cell cycle progression |
| G6PD, TKTL1 | | Pentose phosphate pathway, fatty acids and cholesterol biosynthesis |
| ACL, ME, ACACA, FASN | | Glucose metabolism to lipid synthesis |
| BAP1, PBRM1, SETD2 | H2A, H3 | Pentose phosphate shunt, lipogenesis, AMPK and Krebs cycle activity |
| FBP1 | PDK1, LDHA, GLUT1 | Metabolism |
| | VEGF, VEGFR | Angiogenesis |
| | CXCR4, CXCR7, CX3CR1 | Migration |
| | EPO | Erythropoiesis |
| FH | PHD, VHL, HIF | Energy production, angiogenesis, cell growth, cell proliferation, oxidative stress |
| | KEAP1, Nrf2 | Antioxidant response |
| | AMPK | Fatty acid synthesis, mTOR pathway |
| SDH | PHD, HIF | Energy production |
| PI3K/mTOR | S6K1, 4E-BP1, SREBP-1c, PPARγ, ACACA, FASN, SCD, Lpin1 | Survival, growth, proliferation, motility, metabolism, energy balance, stress response and angiogenesis, adipogenesis |
| MET | RAS-MAPK, PI3K/Akt, STAT3, JNK, EGFR, Plexin-B, CD44, α6β4 integrin | Tumor cell proliferation, survival, invasion and angiogenesis |
| TSC1, TSC2 | LKB1/AMPK/TSC/mTOR, HIF1, VEGF | mRNA translation, protein synthesis and cellular growth |
| FLCN | FNIP1, FNIP2, AMPK, mTOR | mRNA translation, protein synthesis and cellular growth |

Legend: H2A: histone H2A, H3: histone H3, ME: malic enzymes.

Therefore, pVHL is a component of the oxygen and iron sensing pathway that regulates intra-cellular HIF levels, controlling transduction of signals generated by changes in ambient oxygen tension.

Under normoxic conditions, HIF prolyl hydroxylase (PHD) hydroxylates HIF-α on two conserved critical proline residues, enabling HIF-α to bind to pVHL. This process requires molecular oxygen, 2-oxoglutarate, ascorbate and Fe2+ as cofactors. Prolyl-hydroxylation of HIF-α allows its recognition and proteasomal degradation by the E3 ubiquitin ligase complex [10,11]. During hypoxia, or in case of VHL-gene mutations, HIFα subunits accumulates as a result of pVHL inability to determine their destruction, leading to stably up-regulation of hypoxia-response elements (HREs) such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), and the glucose transporter, GLUT1 [12]. HIF transcriptional activity, especially HIF2α activity [13], mediates angiogenesis, epithelial–mesenchymal transition (EMT), stem-cell maintenance, invasion, metastasis, and resistance to radiation therapy and chemotherapy in kidney cancer [14]. While HIF-1α and HIF-2α demonstrate overlapping effects on angiogenesis and ECM remodeling, they differ in controlling cell metabolism, proliferation, and oncogene activation in human ccRCCs [15]. HIF1α – but not HIF2α – stimulate glycolytic enzyme expression [16], inhibits anabolic biosynthesis by restraining mitochondrial pyruvate consumption [17], and limits cell cycle progression in vitro by post-translationally inhibiting the c-Myc oncoprotein [18]. By contrast, HIF-2α plays a key role in promoting genomic integrity and cell cycle progression by stimulating c-Myc-mediated activation of cyclin D2 (CCND2) and E2F1, and repression of p21 and p27 [19].

Therefore, RCC reliance on aerobic glycolysis can be observed in clear cell RCC carrying mutations on the VHL/HIF pathway (mimicking a hypoxia status with subsequent up-regulation of HIF-responsive genes such as VEGF, PDGF, EGF and glucose transporters GLUT1 and GLUT4).

Targeting the VHL pathway by inhibiting its downstream components, including VEGF and PDGF receptors, represents the fundamental strategy developed for ccRCC treatment. As known, everolimus [20] and temsirolimus [21], which inhibit mTOR which in its turn increases HIF1α gene expression, have reached clinical development. The efficacy of anti-VEGF drugs, including the VEGF-neutralizing antibody bevacizumab [22] and the VEGF-receptor antagonists sunitinib [23], sorafenib [24], pazopanib [25] and axitinib [26], molecularly relies in the over-expression of the HIF-response growth factor VEGF as a consequence of VHL-inactivation.

However, these agents target only a small portion of the downstream genes regulated by HIF. Approaches to block HIFα transcriptional activity so as to affect all of the genes regulated by HIF are under evaluation to provide a more effective anticancer therapy. The topoisomerase 1 inhibitor, topotecan, repressing HIF1α-mediated transcription [27], is a potential effective treatment. Despite the initial failure of topotecan monotherapy [28], the pre-clinical data of its combination with anti-angiogenic TKIs (such as pazopanib) look promising [29]. Small molecules, like Acriflavine that directly binds to HIF1α and HIF2α, can inhibit HIF1 dimerization with potent inhibitory effects on tumor growth and vascularization [30]. Despite the difficulties to directly inhibit HIF itself, several agents have been developed to indirectly down-regulate HIF, including mTOR inhibitors, HSP90 inhibitors and HDAC inhibitors [31]. Moreover, pVHL loss might influence the EGFR signaling pathway via TGFα and its receptor EGFR up-regulation [32], however no clinical activity has been observed with EGFR inhibitors [33].

## *Energy sensing pathways*

### *Warburg's effect*

Glucose physiologically can follow three main metabolic pathways: (1) isomerization to fructose-6-phosphate and subsequent degradation during glycolysis to form pyruvate; (2) oxidation to 6-phospho-gluconate and conversion in the pentose phosphates; (3) transformation into glucose-1-phosphate to form glycogen. In multicellular organisms in normoxic conditions, glucose is metabolized to carbon dioxide by oxidation of glycolytic pyruvate in the mitochondrial tricarboxylic acid (TCA) cycle, producing NADH, which then fuels oxidative phosphorylation to maximize adenosine 5-triphosphate (ATP) production, with marginal production of lactate. However, glycolysis is the only process of glucose utilization under hypoxia, leading to large amounts of lactic acid as terminal product. Unlike normal tissues, the anaerobic degradation of glucose (glucose fermentation into lactate) even in the presence of adequate oxygen availability for mitochondrial oxidative phosphorylation is the hallmark of cancer cells metabolism,

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