

A false sense of hypoxia: Pro-tumorigenic VHL-HIF2 α dysregulation in clear cell renal cell carcinoma (Review)

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Abstract. Clear cell renal cell carcinoma (ccRCC) is the most prevalent subtype of renal cell carcinoma and is characterized by bi-allelic inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene. Loss of VHL leads to stabilization of hypoxia-inducible factor α (HIF α), creating a persistent pseudohypoxic or ‘false sense of hypoxia’ state in which HIF signalling remains active despite adequate oxygen tension. Within this context, HIF2 α functions as the dominant oncogenic isoform and drives angiogenesis, metabolic rewiring, tumor microenvironment remodeling and modulation of immune responses. In parallel, epigenetic reprogramming and super-enhancer-driven transcriptional programs reinforce ccRCC growth and progression. In this review, these molecular and epigenetic mechanisms were integrated around the VHL-HIF2 α pseudohypoxia framework with particular emphasis on pathological angiogenesis, metabolic reprogramming, tumor heterogeneity and super-enhancer biology. Furthermore, current therapeutic strategies, including VEGF/VEGFR-targeted agents, HIF2 α inhibitors, mTOR pathway inhibitors and immune checkpoint blockade, were presented, together with mechanisms that limit response durability. Finally, emerging precision-oncology approaches aimed at resolving intratumor heterogeneity, identifying predictive biomarkers and exploiting tumor- and microenvironment-specific vulnerabilities to overcome therapeutic resistance were discussed.

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1. Introduction

Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cell carcinoma, accounting for ~75-80% of kidney cancer cases (1). Clinical management is stage-dependent, with surgery representing the mainstay of treatment for localized disease, whereas advanced or metastatic disease is managed using systemic therapies including anti-angiogenic agents, mTOR pathway inhibitors, immune checkpoint inhibitors and hypoxia-inducible factor (HIF)2 α -targeted therapies (2). However, owing to its asymptomatic presentation at the early stage, a substantial proportion of patients already present with metastatic disease at diagnosis (1). Advanced stage ccRCC is highly aggressive, with frequent metastasis to the lung, lymph nodes and bones, and is associated with high mortality due to poor responsiveness to therapies (3). Despite significant advances in developed therapeutic strategies over recent decades, efficacy and durable treatment responses remain limited, rendering this highly heterogeneous malignancy a persistent clinical challenge (4).

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At the molecular level, ccRCC is defined by recurrent alterations on chromosome 3p, most notably bi-allelic inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene (5,6). Loss of VHL function disrupts the cellular oxygen sensor by causing constitutive stabilization of its downstream target, HIF α , even under normoxic conditions (7). This aberrant activation of the VHL-HIF α axis drives pro-oncogenic transcriptional programs that promote ccRCC growth and progression (8). In parallel, accumulating evidence also implicates epigenetic dysregulation and super-enhancer-driven transcriptional networks as critical drivers of disease progression (9,10). In this review, current knowledge of the molecular and epigenetic alterations underpinning ccRCC pathogenesis was synthesized, existing therapeutic strategies and their limitations were evaluated and emerging opportunities to improve patients' stratification and treatment outcomes were discussed. Conceptually, VHL loss therefore converts a normally reversible physiological hypoxia response into a constitutive oncogenic program: The tumor behaves as though it remains hypoxic even when oxygen is available. This pseudohypoxic 'false sense of hypoxia' provides the unifying framework for the angiogenic, metabolic, epigenetic and microenvironmental alterations discussed throughout this review.

2. Clinical overview and molecular landscape of ccRCC

According to GLOBOCAN, an estimated 434,840 new cases of kidney cancer were diagnosed worldwide in 2022, making it the 14th most common cancer, with ~155,953 associated deaths (11). ccRCC, which originates from proximal tubular epithelial cells, is the most prevalent kidney cancer subtype that contributes to ~75-80% of all cases (1). The term 'clear cell' arises from the distinctive, clear, glass-like cytoplasm of ccRCC, which results from the accumulation of cytoplasmic lipids and glycogen (12). Other hallmark features of ccRCC include increased angiogenesis leading to tumor hypervascularization and frequent mTOR signaling pathway hyperactivation, which together support tumor growth and survival (13,14). Clinically, the management of ccRCC is highly dependent on the stage at diagnosis, with localized tumors typically treated by partial or radical nephrectomy, which can be curative when the disease is detected early (1).

However, a substantial proportion of patients present with advanced or metastatic disease, for which surgical intervention alone is insufficient. Metastatic spread most commonly involves the lungs, lymph nodes and bones (3). ccRCC is associated with an unfavorable prognosis, as ~20-30% of patients with localized ccRCC experience disease recurrence following curative surgery and the tumor exhibits notable resistance to conventional chemotherapy and radiotherapy (15). Over the past 2 decades, advances in molecular and immunological understanding have driven the development of targeted therapies, including tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors, which have significantly improved clinical outcomes (16). Nevertheless, a substantial proportion of patients exhibit primary resistance or ultimately develop acquired resistance, leading to disease progression or relapse (4,17).

Therapeutic inefficacy in ccRCC is multifactorial and driven in part by pronounced intratumor heterogeneity. This is characterized by the coexistence of genetically and

phenotypically distinct subclones within the same tumor that exhibit differential sensitivity to treatment. Multiregional sequencing studies have demonstrated branched evolutionary trajectories in ccRCC, in which truncal alterations are shared across tumor regions, while later driver events emerge in distinct subclonal populations (5,18). In parallel, dynamic remodeling of the tumor microenvironment (TME) facilitates immune evasion, sustains angiogenesis and promotes adaptive resistance to therapy (4). Persistent accumulation of HIF α due to VHL loss further supports tumor survival under both hypoxic and pseudohypoxic conditions by driving angiogenic and metabolic reprogramming, thereby facilitating adaptation to oxygen and nutrient stress during tumor progression and therapeutic pressure (19). Collectively, the interplay between aberrant angiogenesis, metabolic adaptation, TME remodeling and intratumor heterogeneity underpins resistance to current therapies and underscores the biological and clinical complexity of ccRCC. These challenges highlight the urgent need for more effective and durable therapeutic strategies capable of overcoming treatment resistance.

At the molecular level, ccRCC is defined by bi-allelic inactivation of the VHL tumor suppressor gene, which represents a key truncal event and initiating driver in ccRCC pathogenesis (18). VHL is a classic two-hit tumor suppressor that conforms to Knudson's hypothesis of cancer development (20). In sporadic ccRCC, bi-allelic loss of VHL most commonly arises through deletion or loss of heterozygosity of one allele, commonly as part of chromosome 3p loss, followed by mutation or other inactivation of the remaining allele (18,21). In addition to genetic alterations, epigenetic silencing via hypermethylation of the VHL promoter has also been reported and contributes to loss of VHL expression in a subset of ccRCC cases (22). Collectively, these mechanisms result in loss of VHL function in ~90% of sporadic ccRCCs, establishing VHL inactivation as the hallmark gatekeeper alteration of this malignancy. Beyond sporadic disease, germline inactivation of the VHL gene underlies VHL syndrome, a hereditary cancer predisposition disorder characterized by the development of ccRCC and other highly vascular tumors (7). The implications of VHL loss and consequent HIF α accumulation, which are central to driving ccRCC initiation, are discussed in detail in chapter 4.

Beyond VHL, the molecular landscape of ccRCC is also characterized by recurrent mutations in additional tumor suppressor genes, particularly those involved in chromatin remodeling and epigenetic regulation. Among these, polybromo-1 (PBRM1) represents the second most frequently mutated gene in ccRCC following VHL loss (22,23). *PBRM1* encodes BRG1-associated factor 180, a core subunit of the SWI/SNF chromatin remodeling complex. Its loss or inactivation has been associated with higher tumor grade and poorer clinical outcomes in patients with ccRCC (24). However, the prognostic significance of PBRM1 alterations appears to be context dependent, with differences reported between localized and advanced disease and according to treatment exposure (25,26). Other commonly mutated tumor suppressors include SET domain containing 2 (*SETD2*) and BRCA1-associated protein 1 (*BAP1*), which encode a histone H3 lysine 36 trimethyl transferase and a histone deubiquitinase, respectively (27). Both *SETD2* and *BAP1* conform to

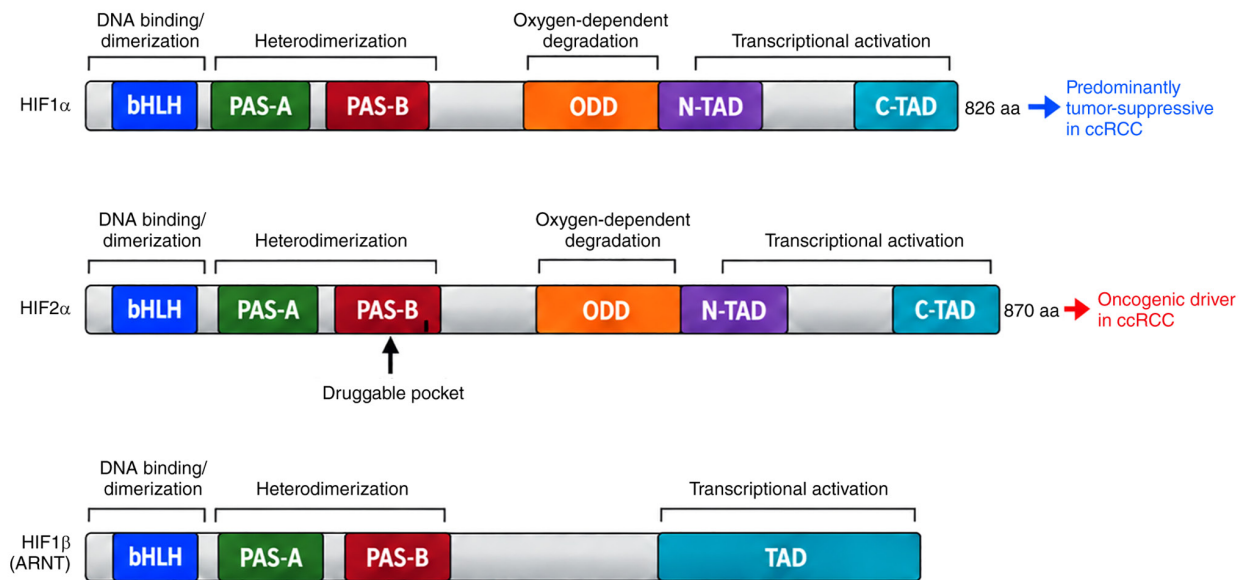


Figure 1. Domain organization and functional divergence of HIF proteins in ccRCC. HIF1α and HIF2α share conserved structural domains but differ in protein length and biological function. In ccRCC, HIF1α is more commonly associated with growth-restraining effects, whereas HIF2α functions as a major oncogenic driver. HIF1β (ARNT) serves as the constitutive heterodimerization partner. HIF, hypoxia-inducible factor; ccRCC, clear cell renal cell carcinoma; HIF1α, hypoxia-inducible factor 1 alpha; HIF2α, hypoxia-inducible factor 2 alpha; HIF1β, hypoxia-inducible factor 1 beta; ARNT, aryl hydrocarbon receptor nuclear translocator; bHLH, basic helix-loop-helix; PAS, Per-Arnt-Sim; PAS-A, Per-Arnt-Sim domain A; PAS-B, Per-Arnt-Sim domain B; ODD, oxygen-dependent degradation domain; N-TAD, N-terminal transactivation domain; C-TAD, C-terminal transactivation domain; TAD, transactivation domain.

Knudson's two-hit hypothesis, with BAP1 mutations in particular being enriched in high-grade and clinically aggressive ccRCC (28).

Recent whole-genome sequencing further confirmed the predominance of these alterations, with mutations reported in *VHL* (~80%), *PBRM1* (~50%), *SETD2* (~18%) and *BAP1* (~12%) in ccRCC (29). The high prevalence of alterations affecting chromatin remodeling complexes and chromatin-modifying enzymes underscores the central role of epigenetic deregulation alongside VHL loss in driving ccRCC pathogenesis. Notably, *VHL*, *PBRM1*, *SETD2* and *BAP1* are all located on the short arm of chromosome 3 (3p), placing them in close physical proximity within a single chromosomal region. As a result, loss of heterozygosity of chromosome 3p is a defining early genomic event in ccRCC and is estimated to occur in up to 90% of cases (21). This chromosomal loss simultaneously removes one allele of several major ccRCC tumor suppressors, thereby creating a permissive genomic background in which subsequent second-hit alterations can drive divergent evolutionary trajectories.

3. VHL-HIF oxygen-sensing pathway and the molecular basis of pseudohypoxia

The VHL protein (pVHL) functions as the substrate recognition component of an E3 ubiquitin ligase complex that is commonly referred to as the VHL complex. This complex includes Elongin B, Elongin C, Cullin-2 and RING-box protein 1, which mediates ubiquitin-dependent proteasomal degradation of specific target proteins (7). pVHL is present in both the cytoplasm and nucleus and undergoes dynamic nucleocytoplasmic shuttling, which contributes to the spatial regulation of its substrate interactions (30). Among the known substrates of pVHL, transcription factor HIFα is the most

well-characterized and directly linked to the tumor suppressor function of the VHL complex (31). A total of three HIFα isoforms have been identified to date, of which HIF1α and HIF2α are the most extensively studied (32).

The transcriptional activity of HIF depends on the formation of a heterodimer between the oxygen-sensitive HIFα subunit and the constitutively expressed HIF1β (also known as ARNT). Structurally, HIF1α and HIF2α share a conserved domain architecture comprising the following: i) An N-terminal basic helix-loop-helix (bHLH) domain responsible for DNA binding, ii) Per-Arnt-Sim (PAS) domains that mediate protein dimerization, iii) an oxygen-dependent degradation domain (ODD), and N- and C-terminal transactivation domains (N-TAD and C-TAD) that regulate transcriptional activity (32,33). By contrast, the constitutively expressed HIF1β subunit contains the bHLH and PAS dimerization domains but lacks the ODD domain. HIF1β also contains a C-TAD even though HIFα provides the principal oxygen-regulated transcriptional activity of the heterodimer (34). Hence, unlike HIFα, its abundance is therefore not directly regulated by oxygen-dependent proteolysis. The structural organization and domain composition of HIF1α, HIF2α and HIF1β are illustrated in Fig. 1.

Although HIF1α and HIF2α share a conserved domain organization, they are distinct proteins that differ in amino acid sequence and protein length. Their DNA-binding, dimerization and C-TAD domains exhibit ~83, 70 and 67% sequence similarity, respectively (35). Although both are oxygen-sensitive transcription factors regulated by the same core oxygen-sensing machinery, differences within their oxygen-dependent degradation domains and in hydroxylase preference contribute to distinct regulation and stability. HIF1α is hydroxylated at Pro402 and Pro564, whereas HIF2α is hydroxylated at Pro405 and Pro531 with prolyl hydroxylase

domain 2 (PHD2) showing preferential activity toward HIF1 α (35). These functional differences are particularly important in ccRCC and are discussed in Chapter 4.

Although HIF1 α and HIF2 α use the same oxygen-sensing pathways, they are not regulated to the same extent. In addition to the preferential activity of PHD2 toward HIF1 α described above, PHD1 and PHD3 can contribute more substantially to HIF2 α regulation, although their relative contributions are context-dependent (35,36). Factor inhibiting HIF (FIH) can hydroxylate both HIF1 α and HIF2 α but HIF1 α is more efficiently hydroxylated than HIF2 α . This difference can affect p300/CREB-binding protein (CBP) recruitment and transcriptional activity (37,38). Thus, within the shared oxygen-sensing pathway, the two isoforms differ in their sensitivity to the hydroxylases that control protein stability and transcriptional activity rather than through separate activation and inactivation pathways. These isoform-specific differences occur within the shared oxygen-dependent regulatory pathway described below.

Upon heterodimerization, the HIF complex drives hypoxia-responsive transcriptional programs that facilitate cellular adaptation and survival under low-oxygen conditions, including angiogenesis, metabolic reprogramming and enhanced stress resistance (39,40). Unlike the oxygen-independent HIF1 β , the HIF α subunit is intrinsically unstable under normoxic conditions and therefore represents the rate-limiting component of the transcriptionally active HIF complex. Under normoxic conditions, when hypoxia-driven transcriptional programs are not required, specific proline residues within the ODD domain of HIF α are hydroxylated by prolyl hydroxylase enzymes (41). PHD activity requires molecular oxygen, Fe²⁺ and 2-oxoglutarate, thereby directly coupling HIF α stability to cellular oxygen availability. The prolyl-hydroxylated HIF α is subsequently recognized by the VHL complex, which catalyzes its ubiquitination and targets it for proteasomal degradation (31,41). In parallel, FIH hydroxylates a conserved asparagine residue within the HIF α C-TAD under normoxic conditions, thereby limiting recruitment of the transcriptional coactivators p300/CBP and providing an additional oxygen-sensitive layer of HIF regulation (42).

However, reduced oxygen availability under hypoxic conditions impairs PHD enzyme activity and prevents prolyl hydroxylation of HIF α . Consequently, HIF α escapes recognition by the VHL complex, accumulates and heterodimerizes with HIF1 β to form a transcriptionally competent HIF complex (7,41). This complex translocates to the nucleus and binds hypoxia response elements, with the core consensus sequence 5'-[A/G]CGTG-3' within promoters and other regulatory regions of target genes, initiating gene expression programs that facilitate cellular adaptation to hypoxia (43). Through this tightly regulated mechanism, HIF α accumulation is prevented under normal oxygen tension, thereby suppressing aberrant activation of hypoxia-inducible transcriptional programs.

In VHL-deficient ccRCC, this oxygen-sensing checkpoint is uncoupled from the actual oxygen tension. HIF-dependent transcription therefore remains constitutively active despite adequate oxygen availability, creating a molecular 'false sense of hypoxia' or pseudohypoxic state. This persistent pseudohypoxia provides the central biological framework through which VHL loss promotes oncogenic signaling in

ccRCC (19). Importantly, this converts a normally transient and reversible physiological response to low oxygen into a constitutively active transcriptional program that promotes tumor-associated angiogenic, metabolic and survival pathways. The contrasting regulation of HIF2 α under normoxic conditions and VHL-loss-driven pseudohypoxia in ccRCC is illustrated in Fig. 2.

4. VHL loss establishes HIF2 α -dominant oncogenic signaling in ccRCC

In the context of ccRCC, bi-allelic inactivation of VHL disrupts the oxygen-dependent regulation of HIF α , leading to the constitutive stabilization of HIF α even under normoxic conditions. This pathological accumulation of HIF α aberrantly activates the hypoxia-responsive transcriptional programs, thereby promoting ccRCC pathogenesis through the induction of multiple pro-tumorigenic downstream targets (39). Among these targets, vascular endothelial growth factor A (VEGFA) is a central mediator of angiogenesis and has been implicated in the tumorigenesis of multiple cancer types (44). In parallel, sustained HIF α signaling promotes metabolic reprogramming characterized by increased expression of glycolytic enzymes and reduced reliance on mitochondrial oxidative metabolism, contributing to the aerobic glycolytic phenotype commonly referred to as the Warburg effect (45). Additional well-established HIF α target genes involved in ccRCC pathogenesis include cyclin D1 (CCND1), which drives cell cycle progression through activation of cyclin-dependent kinases 4 and 6 (46), and C-X-C chemokine receptor type 4, a key regulator of tumor cell migration and metastasis (47). Consistent with these oncogenic outputs, paired box 8 (PAX8)-lineage conditional mouse models combining VHL loss with either PBRM1 or BAP1 inactivation recapitulate key features of ccRCC, including induction of HIF target genes. Notably, BAP1-deficient tumors exhibit greater mTOR complex 1 (mTORC1) activation than PBRM1-deficient tumors, while additional activation of mTORC1 increases tumor grade in the PBRM1-deficient background (48). These findings support mTORC1 activity as a cooperating determinant of ccRCC progression and tumor grade in the context of VHL deficiency.

As outlined in Chapter 3, transcriptional activation of HIF target genes requires heterodimerization of either HIF1 α or HIF2 α with the HIF1 β subunit. Importantly, accumulating evidence indicates that these HIF α isoforms regulate overlapping yet distinct transcriptional programs, with subsets of genes being preferentially or exclusively controlled by each isoform (49). In ccRCC, multiple studies have demonstrated that HIF2 α functions as a dominant oncogenic driver, whereas HIF1 α may exert tumor-suppressive effects (50-52). Consistent with these functional distinctions, VHL-deficient ccRCC cell lines and clinical tumor samples frequently co-express HIF1 α and HIF2 α or exhibit selective expression of HIF2 α alone (50). A functional study further supported a central oncogenic role for HIF2 α , as short hairpin RNA-mediated knockdown of HIF2 α suppressed tumor formation by VHL-deficient ccRCC cells. Furthermore, expression of a VHL-resistant HIF2 α variant was sufficient to overcome tumor suppression following VHL reconstitution (53). By contrast, normoxic stabilization of HIF1 α alone was insufficient to reproduce the tumorigenic

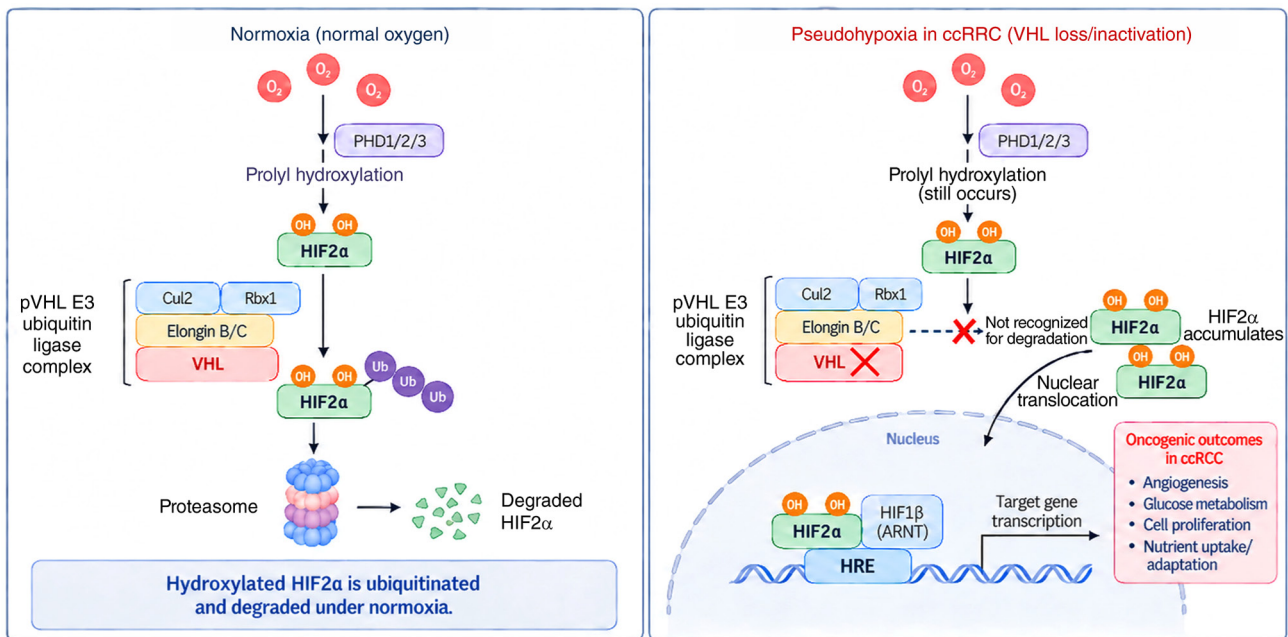


Figure 2. Regulation of HIF2 α under normoxic and pseudohypoxic conditions in ccRCC. Under normoxia, hydroxylated HIF2 α is recognized by the VHL E3 ubiquitin ligase complex and degraded by the proteasome. In VHL-deficient ccRCC, HIF2 α escapes degradation, accumulates and aberrantly activates HIF-dependent transcriptional programs that promote tumor formation and progression. HIF, hypoxia-inducible factor; HIF2 α , hypoxia-inducible factor 2 α ; ccRCC, clear cell renal cell carcinoma; PHD1/2/3, prolyl hydroxylase domain proteins 1, 2 and 3; Cul2, cullin 2; Rbx1, RING-box protein 1; Elongin B/C, elongin B and elongin C; Ub, ubiquitin; HIF1 β , hypoxia-inducible factor 1 β ; ARNT, aryl hydrocarbon receptor nuclear translocator; HRE, hypoxia-response element; VHL, von Hippel-Lindau.

phenotype associated with VHL loss (54). Collectively, these findings establish pathological HIF2 α stabilization as a key oncogenic consequence of VHL loss in ccRCC, although the relative contributions of HIF1 α and HIF2 α remain dependent on the molecular and cellular context. The broader contributions of the VHL-HIF2 α axis to angiogenesis, metabolic reprogramming and mTORC signaling in ccRCC are discussed in the following subsections.

The opposing biological roles of HIF1 α and HIF2 α in ccRCC are partly explained by their differential effects on c-Myc. HIF1 α can antagonize c-Myc activity and promote cell-cycle arrest through p21, whereas HIF2 α enhances c-Myc transcriptional activity and supports cell proliferation (50,55). This functional divergence extends beyond c-Myc, as HIF2 α preferentially regulates several pro-tumorigenic genes in VHL-deficient RCC, including *CCND1*, *TGFA* and *VEGFA*, whereas HIF1 α preferentially activates distinct targets such as the pro-apoptotic gene *BCL2* interacting protein 3 (49). Collectively, these transcriptional differences are consistent with a more growth-restraining role for HIF1 α in ccRCC, whereas HIF2 α preferentially supports pro-proliferative and pro-angiogenic programs (51). HIF1 α is encoded by *HIF1A* at chromosome 14q23.2, whereas HIF2 α is encoded by endothelial PAS domain protein 1 (*EPAS1*) at chromosome 2p21. The recurrent loss of 14q encompassing *HIF1A*, together with reported loss-of-function alterations of this gene, is consistent with selective pressure against HIF1 α activity during ccRCC evolution (52). Nevertheless, the functional dichotomy between HIF1 α and HIF2 α is not absolute, as their effects on tumor development, metabolism and inflammation vary according to the cellular and genetic

background (56). These isoform-specific differences have direct therapeutic implications. The preferential dependence of VHL-deficient ccRCC on HIF2 α provides a biological rationale for selective HIF2 α inhibition rather than indiscriminate suppression of HIF signaling (57). Thus, although HIF2 α represents the dominant oncogenic HIF isoform in ccRCC, the biological consequences of HIF1 α and HIF2 α activity remain genotype- and context-dependent.

VHL-HIF2 α -VEGFA signaling and pathological angiogenesis. Angiogenesis is defined as the formation of new blood vessels from pre-existing vasculature, which is essential for tumor growth and metastatic dissemination by ensuring sustained oxygen and nutrient supply. Tumors associated with VHL disease are characteristically highly vascularized, reflecting dysregulated HIF-dependent angiogenic signaling. In primary renal tumors, HIF2 α expression is associated with increased VEGFA levels and greater microvessel density, while VEGFA is preferentially regulated by HIF2 α in VHL-deficient RCC cells (49,58). Together, these findings support the VHL-HIF2 α -VEGFA axis as a major driver of the hypervascular phenotype of ccRCC. Under normal physiological conditions, angiogenesis is tightly coordinated through mechanisms that regulate endothelial sprouting and vessel maturation, including Dll4-Notch control of tip and stalk cell specification and platelet-derived growth factor (PDGF)-B/PDGF receptor (PDGFR) β -mediated pericyte recruitment (59). In ccRCC, sustained VEGFA production driven by VHL loss and HIF2 α accumulation perturbs this coordinated vascular program, providing the basis for the abnormal tumor vasculature characteristic of the disease.

The angiogenic effects of VEGFA are primarily mediated through its interaction with VEGF receptors (VEGFRs), particularly VEGFR1 and VEGFR2. Among these, VEGFR2 serves as the principal signaling receptor, driving mobilization of endothelial progenitor cells, endothelial cell proliferation, migration and vascular permeability (60). The functional importance of VEGFR2 signaling in ccRCC is supported by a study showing that sunitinib, a multi-targeted TKI that inhibits VEGFR2, suppresses renal tumor growth predominantly through its effects on tumor endothelial cells. This includes inhibition of VEGFR2 signaling and reduction of tumor microvessel density, rather than through direct cytotoxic effects on ccRCC cells (61). At the molecular level, phosphorylation of VEGFR2 at Y1173 in mice, corresponding to Y1175 in humans, activates phospholipase C (PLC) γ -dependent signaling. Activation of PLC γ subsequently promotes Ca²⁺/protein kinase C-dependent activation of endothelial nitric oxide synthase, followed by Src activation and phosphorylation of vascular endothelial cadherin, thereby destabilizing endothelial adherens junctions and increasing vascular permeability (62). Importantly, this mechanism has direct clinical relevance to ccRCC, as high endothelial PLC γ expression in human ccRCC has been associated with increased angiogenic activity and poorer clinical outcome (62).

In an *in vivo* study, attenuation of VEGFR2-PLC γ signaling using VEGFR2^{Y1173F/+} mutant mice resulted in stabilization of the endothelial barrier, reduced vascular leakage and decreased infiltration of immunosuppressive cell populations, including B cells and regulatory T cells. These vascular changes were accompanied by enhanced antitumor immunity and improved responses to chemotherapy and immunotherapy (62). In ccRCC, VEGFR2-PLC γ signaling can therefore be viewed as a downstream effector of the VHL-HIF2 α -VEGFA axis, in which VHL loss and HIF2 α accumulation sustain VEGFA expression and VEGFR2 activation. Collectively, this links pseudohypoxic angiogenic signaling not only to vascular dysfunction but also to remodeling of the immune microenvironment.

The considerable progress in the understanding of ccRCC pathogenesis, particularly the central role of the VHL-HIF2 α -VEGFA axis, has provided a strong rationale for the development of anti-angiogenic therapies. Several VEGFA/VEGFR-targeted agents have received approval for the treatment of advanced ccRCC, including TKIs such as sunitinib, sorafenib and lenvatinib, as well as the VEGFA-neutralizing monoclonal antibody bevacizumab (57). However, their long-term efficacy is frequently limited by the emergence of resistance. Importantly, resistance to VEGF-targeted therapy does not necessarily indicate loss of angiogenic dependence. Instead, ccRCC cells can activate alternative pro-angiogenic pathways that restore tumor vascularization despite continued VEGFR inhibition. For example, increased IL-8 expression has been shown to mediate sunitinib resistance in ccRCC xenografts, whereas IL-8 neutralization restored sensitivity to treatment (63). Prolonged VEGF-targeted therapy can similarly promote the production of alternative endothelial growth factors that support VEGF-independent tumor revascularization (64), which provide a rationale for combination approaches targeting complementary angiogenic pathways. Furthermore, the functional interplay between

angiogenesis and immune regulation has provided a biological basis for combining VEGFR-targeted TKIs with immune checkpoint inhibitors. For instance, pembrolizumab plus axitinib improved survival outcomes compared with sunitinib in the phase III KEYNOTE-426 trial (65), while nivolumab plus cabozantinib similarly demonstrated superior efficacy over sunitinib in CheckMate 9ER (66). Together, these findings demonstrate that the VHL-HIF2 α -VEGFA axis influences not only tumor vascularization but also the broader TME, thereby providing a mechanistic basis for contemporary therapeutic strategies that simultaneously target angiogenic and immune pathways in advanced ccRCC.

VHL-HIF2 α -driven lipid metabolic reprogramming. In ccRCC, loss of VHL function and accumulation of HIF2 α are closely linked to metabolic reprogramming (67). A defining consequence of this metabolic shift is the extensive accumulation of neutral lipids and lipid droplets that contributes to the characteristic clear-cell morphology. Rather than representing a passive histological feature, this lipid-rich phenotype represents a functional metabolic adaptation that supports ccRCC growth and survival. Mechanistically, HIF signalling suppresses mitochondrial fatty acid β -oxidation by repressing carnitine palmitoyl transferase 1A (CPT1A), a rate-limiting component of mitochondrial fatty acid transport (68). In VHL-deficient ccRCC cells, CPT1A is directly repressed by both HIF1 α and HIF2 α , thereby limiting fatty acid transport into mitochondria and redirecting fatty acids toward lipid-droplet storage. Importantly, restoration of CPT1A reduced lipid deposition and suppressed tumor growth (68). HIF2 α also promotes lipid storage through upregulation of the lipid-droplet coat protein perilipin 2 (PLIN2). HIF2 α -dependent PLIN2 expression maintains lipid-droplet formation and protects ccRCC cells from cytotoxic endoplasmic reticulum stress, thereby supporting tumor-cell viability under metabolic stress (69).

In addition to suppressing lipid catabolism and promoting lipid storage, HIF2 α activates transcriptional networks that enhance lipid biosynthesis. Super enhancer-driven Kruppel-like factor (KLF)6, partially activated by HIF2 α , regulates multiple genes involved in lipid metabolism. KLF6 additionally induces PDGFB, leading to mTORC1 activation and increased activity of the downstream lipid regulators sterol regulatory element binding transcription factor 1 (SREBP1) and SREBP2 (9). More recently, mediator complex subunit 15 (MED15) was identified as a direct HIF2 α transcriptional target that further promotes SREBP-dependent lipid synthesis in ccRCC. MED15 acts as a coactivator of SREBP1 and SREBP2 and enhances the expression of lipogenic enzymes, including fatty acid synthase, acetyl-CoA carboxylase 1, ATP citrate lyase (ACLY) and stearyl-CoA desaturase 1, partly through activation of PLK1/AKT signaling (70). HIF2 α depletion reduced lipid accumulation and malignant phenotypes, whereas MED15 re-expression partially restored lipid deposition, proliferation, migration and invasion, supporting a functional HIF2 α -MED15-SREBP axis in ccRCC lipid metabolism (70).

The impact of VHL-HIF signaling on lipid metabolism also extends to cholesterol homeostasis. Analyses of primary human ccRCC demonstrated that VHL mutation promotes accumulation of cholesteryl esters through HIF α -dependent activation of the PI3K/AKT/mTOR/SREBP pathway (71).

Restoration of wild-type VHL or suppression of HIF2 α reduced cholesteryl ester accumulation, whereas pharmacological inhibition of cholesterol esterification impaired ccRCC growth *in vitro* and in patient-derived xenografts (71). Collectively, these findings indicate that VHL-HIF2 α -driven lipid reprogramming in ccRCC involves coordinated suppression of fatty acid oxidation, enhancement of lipid storage and activation of lipid biosynthetic pathways. These interconnected metabolic adaptations contribute to the lipid-rich phenotype of ccRCC while supporting tumor growth and survival.

Furthermore, phospholipid remodeling represents another component of HIF2 α -driven metabolic reprogramming in ccRCC. Lysophosphatidylcholine (LPC) acyltransferase 1 (LPCAT1) catalyzes the conversion of LPC to PC. ccRCC tissues exhibit increased LPCAT1 expression together with corresponding alterations in LPC and PC profiles. Elevated LPCAT1 expression is associated with adverse clinicopathological features and poorer overall survival (OS), while LPCAT1 depletion reduces PC levels, proliferation and invasion as well as inducing G0/G1 cell-cycle arrest (72). More recent studies have directly linked LPCAT1 to HIF2 α signaling. HIF2 α transcriptionally activates LPCAT1, which subsequently promotes triglyceride accumulation and lipid-droplet formation. Mechanistically, LPCAT1 regulates the F-box and WD repeat domain containing 7-ACLY axis, thereby sustaining fatty acid and triglyceride synthesis and tumor growth (73). In addition, HIF2 α -induced LRRK2 stabilizes LPCAT1 and activates AKT/IL-1 β signaling, reducing sensitivity to VEGFR-targeted therapy and programmed cell death 1 (PD-1) blockade (74). Collectively, these findings position LPCAT1 as an important downstream mediator of HIF2 α -dependent lipid remodeling that contributes to both ccRCC progression and therapeutic resistance.

PI3K/AKT/mTORC1 signaling downstream of pseudohypoxia. The PI3K/AKT/mTOR pathway is frequently dysregulated in ccRCC and represents an important point of convergence between oncogenic growth-factor signaling and VHL-HIF2 α -driven pseudohypoxia. The relevance of this pathway to ccRCC is supported by large-scale genomic analyses showing recurrent alterations in PI3K/AKT/mTOR pathway components, including mTOR and phosphatase and tensin homolog (PTEN) (22). Beyond these genomic alterations, VHL-HIF2 α signaling can directly promote mTORC1 activity. Elorza *et al* (75) demonstrated that HIF2 α transcriptionally induces the amino acid transporter solute carrier family 7 member 5 (SLC7A5), thereby sustaining amino acid-dependent mTORC1 activity under nutrient-limited conditions. In VHL-deficient RCC cells, HIF2 α or SLC7A5 depletion reduced mTORC1 signaling, while SLC7A5 was required for efficient growth of VHL-deficient xenografts (75).

The relationship between VHL loss and mTORC1 activation is not exclusively HIF-dependent. Ganner *et al* (76) demonstrated that VHL directly promotes regulatory associated protein of mTORC1 (RAPTOR) ubiquitination and proteasomal degradation, thereby limiting the abundance of this essential mTORC1 component. Accordingly, VHL deficiency increased RAPTOR abundance and enhanced downstream mTORC1 activity in ccRCC cells. Pharmacological inhibition of either HIF1 α or HIF2 α did not

alter RAPTOR abundance, supporting a HIF-independent mechanism through which VHL loss can enhance mTORC1 activity (76). In parallel, several HIF2 α -dependent mechanisms can also promote mTOR signaling. Functional studies showed that repression of either KLF6, partially activated by HIF2 α , or its downstream target PDGFB, reduced mTORC1 activity, whereas recombinant PDGFB restored mTORC1 signaling in KLF6-depleted ccRCC cells (9). Consistent with these findings, studies using primary human ccRCC cells showed that restoration of wild-type VHL or suppression of HIF2 α reduced mTOR activity (71). Collectively, VHL loss can enhance PI3K/AKT/mTOR signaling through both HIF-independent regulation of mTORC1 components and HIF2 α -dependent signaling programs.

5. Intratumor heterogeneity shapes ccRCC evolution

ccRCC is characterized by extensive intratumoral heterogeneity (ITH) with spatially distinct tumor regions frequently harboring different genetic and phenotypic states. Early multiregional sequencing studies demonstrated branched evolutionary architectures in ccRCC, in which different regions of the same tumor contained distinct subclonal alterations. Gerlinger *et al* (77) subsequently showed that 73-75% of identified driver aberrations were subclonal, while chromosome 3p loss and VHL aberrations represented the principal ubiquitous events across sampled tumor regions. The larger TRACERx Renal study further demonstrated that ccRCC follows multiple evolutionary trajectories, ranging from tumors with early fixation of several driver events and rapid metastatic progression to highly branched tumors containing >10 subclonal driver events (5,18). More recent multi-omics analyses have extended these observations beyond genetic heterogeneity. Analyses of multiregionally sampled tumors revealed that 90% exhibited heterogeneity in at least one molecular or histological feature, while ~50% showed ITH in immune signatures (78). These findings demonstrate that different regions of the same ccRCC can diverge substantially across multiple molecular and microenvironmental dimensions.

Single-cell and spatial profiling have further resolved this heterogeneity at cellular resolution. Multiregional single-cell proteogenomic analysis identified 14 distinct cellular neighborhoods and marked variation in tissue architecture across spatially separated tumor regions (79). Interestingly, immune-cell composition within individual patients remained relatively conserved despite substantial genetic heterogeneity, suggesting that different dimensions of ITH may evolve independently. This phenotypic diversity also extends to tumor metabolism. Hu *et al* (80) identified a de-clear cell differentiated (DCCD) state with distinct metabolic and proliferative properties that coexisted with conventional clear-cell populations in spatially distinct regions of the same tumor. Subclonal DCCD regions exhibited altered metabolic profiles, including reduced fatty-acid abundance, demonstrating that metabolic reprogramming can contribute to spatial ITH (80). Collectively, these studies show that ccRCC ITH extends beyond genetic subclones to encompass distinct cellular, spatial and metabolic states within individual tumors. These states provide a substrate for continued tumor evolution and may contribute to variable therapeutic responses.

6. TME: Linking pseudohypoxia, angiogenesis and immune dysfunction

The TME is inherently heterogeneous and comprises complex interactions between malignant cells, immune and stromal populations, endothelial cells, extracellular matrix and soluble mediators. In ccRCC, this ecosystem is influenced by VHL-HIF-driven pseudohypoxia and its associated angiogenic and metabolic programs. Detailed immune profiling has revealed substantial diversity among tumor-associated macrophage and T-cell populations with specific immune states associated with disease progression and clinical outcome (81). Progression of ccRCC is influenced by an immune-dysfunctional TME, characterized by exhausted T cells and immunosuppressive myeloid populations (82). Among the non-malignant stromal components, cancer-associated fibroblasts (CAFs) have emerged as important regulators of ccRCC progression and therapeutic response (83). Single-cell and spatial analyses identified myofibroblastic CAFs (myCAFs) in close proximity to mesenchymal-like ccRCC cells, with enrichment of myCAFs associated with metastatic disease, poorer survival and primary resistance to immune checkpoint inhibition (83).

Furthermore, T cells, particularly CD8⁺ cytotoxic T lymphocytes, are abundant within the ccRCC microenvironment (84). However, high CD8⁺ T-cell infiltration can paradoxically correlate with poorer prognosis, reflecting the importance of T-cell functional state rather than abundance alone. Single-cell analyses demonstrated enrichment of terminally exhausted CD8⁺ T cells and suppressive macrophages in advanced ccRCC with their coordinated interaction forming an immune dysfunction program associated with poorer outcome (82). Importantly, tumor-intrinsic VHL loss itself may contribute to immune remodeling. In renal tumor models, VHL loss altered tumor-associated macrophage states, reduced lymphocyte activation and diminished response to anti-PD-1 therapy with C-X3-C motif chemokine ligand 1 implicated in VHL-dependent myeloid recruitment (85).

Angiogenic and immune regulation are also closely interconnected in ccRCC. As discussed above, sustained VEGFA-VEGFR signaling downstream of VHL-HIF2 α dysregulation contributes to abnormal tumor vasculature and can influence immune-cell trafficking and function within the TME. Attenuation of endothelial VEGFR2-PLC γ signaling reduced vascular leakage and infiltration of immunosuppressive B cells and regulatory T cells while enhancing antitumor immunity (62). This vascular-immune crosstalk provides a biological rationale for combining immune checkpoint inhibition with VEGFR-targeted therapy, an approach that has demonstrated clinical efficacy in advanced ccRCC (86,87). Nevertheless, substantial TME heterogeneity remains an important challenge for identifying reliable predictive biomarkers and determining therapeutic response.

7. Epigenetic and super enhancer-driven oncogenic transcription in ccRCC

ccRCC is also characterized by extensive reprogramming of its cis-regulatory landscape, arising from both VHL-HIF dysregulation and recurrent alterations in chromatin regulators,

including PBRM1, BAP1 and SETD2. Epigenomic profiling of primary ccRCC tissues showed that VHL loss preferentially alters enhancer activity, with stabilized HIF2 α -HIF1 β recruiting p300 to activate tumor-associated regulatory regions. Super-enhancer profiling identified zinc finger protein 395 as a VHL-regulated transcriptional dependency whose depletion markedly impaired ccRCC growth (10). A recent study further indicated that enhancer reprogramming is established early during tumor development. It was demonstrated that the transition from normal kidney tissue to ccRCC is accompanied by marked changes in chromatin accessibility, enhancer activity and HIF2 α binding, whereas comparatively fewer alterations distinguish localized from metastatic disease (88). The study also identified reactivation of kidney developmental regulatory programs and functionally linked germline susceptibility loci to the ccRCC regulatory landscape (88).

The transcriptional output of HIF2 α is further shaped by renal lineage-specific factors. Patel *et al* (89) showed that HIF2 α is preferentially recruited to PAX8-bound enhancers, including a pro-tumorigenic enhancer regulating *CCND1* expression. PAX8 binding at this enhancer was also influenced by the ccRCC susceptibility variant rs7948643, illustrating how somatic VHL loss, lineage-specific transcriptional programs and inherited genetic variation can converge at regulatory DNA elements (89). More recently, Naas *et al* (90) identified a ccRCC-activated enhancer controlling *EPAS1* expression itself. This enhancer depends on HIF and renal lineage factors, PAX8 and hepatocyte nuclear factor 1 β , thereby establishing a feed-forward circuit that sustains HIF2 α expression and tumor growth (90).

Beyond lineage-specific enhancer regulation, secondary alterations in chromatin regulators can further reshape the ccRCC regulatory landscape. Single-nucleus profiling showed that BAP1-mutant tumors exhibit widespread loss of chromatin accessibility whereas PBRM1 mutations are generally associated with increased accessibility. Notably, BAP1 alterations affected ~5-fold more accessible chromatin regions than PBRM1 alterations, with corresponding changes in gene expression (91). This epigenetic plasticity can also expand the metastatic output of VHL-HIF signalling. Loss of polycomb repressive complex 2-dependent trimethylation of lysine 27 on histone H3 enables HIF-driven C-X-C motif chemokine receptor 4 expression, while DNA demethylation permits HIF-dependent cytohesin 1 interacting protein activation, promoting invasion and survival (92). Subsequent work showed that metastatic ccRCC cells can also co-opt lineage-inappropriate enhancer programs, including a functionally important enhancer cluster jointly regulated by HIF2 α and NF- κ B to promote metastatic competence (47).

Super-enhancer-driven transcriptional programs provide an additional link between epigenetic plasticity, metabolism and tumor progression in ccRCC. KLF6 is driven by one of the strongest super-enhancers in ccRCC and partially activated by HIF2 α . KLF6 subsequently promotes PDGFB-mTORC1 signaling and lipid-metabolic programs that support tumor growth (9). Separately, loss of methyltransferase 14, N6-adenosine-methyltransferase non-catalytic subunit leads to accumulation of the chromatin-remodelling factor bromodomain PHD finger transcription factor, which strengthens super-enhancer activity at genes including enolase 2 and

SRC proto-oncogene, non-receptor tyrosine kinase. Increased expression of these genes promotes glycolytic reprogramming and contributes to metastatic progression in ccRCC (93). Super-enhancer remodelling also contributes to inflammatory signaling in ccRCC. DNA demethylation activates super-enhancers associated with CXC chemokines and other inflammatory genes, promoting neutrophil recruitment and facilitating lung metastasis (94).

Functional studies further support the dependence of ccRCC super-enhancers on chromatin-associated co-regulators. Zamberi *et al* (95) showed that inhibition of bromodomain containing 4 or p300 reduced acetylation of lysine 27 on histone H3 at the KLF6 super-enhancer, decreased KLF6 expression and impaired ccRCC cell growth. Consistent with this, bromodomain and extraterminal domain (BET) inhibition has also been shown to suppress inflammatory transcriptional programs and metastatic progression in ccRCC (94), providing a further rationale for therapeutically disrupting enhancer-associated transcriptional machinery. However, clinical translation remains limited by modest single-agent activity, treatment-related toxicities and the absence of validated predictive biomarkers (96). Early-phase trials of pan-BET inhibitors highlight important translational limitations. Birabresib was associated with dose-limiting grade 3/4 thrombocytopenia and gastrointestinal toxicity with objective responses largely restricted to nuclear protein in testis (NUT) midline carcinoma (97). Molibresib similarly caused thrombocytopenia, gastrointestinal adverse events, anemia and fatigue with responses again concentrated in NUT carcinoma (98). The BAY 1238097 phase I study was terminated early because dose-limiting vomiting, headache and back pain occurred before therapeutically effective exposure was achieved and no objective responses were observed (99). Although these studies were not conducted in ccRCC, they suggest that the modest single-agent activity of BET inhibitors may reflect a narrow therapeutic window, tumor heterogeneity, adaptive transcriptional compensation and the lack of biomarkers for selecting BET-dependent tumors. These limitations suggest that enhancer-directed therapies may be more effective in biomarker-selected patients or in rational combination strategies rather than as unselected monotherapy.

8. Therapeutic strategies in ccRCC

HIF2 α -targeted therapy: Clinical progress and resistance. HIFs were long considered difficult to drug because they lack intrinsic enzymatic activity and rely on protein-protein interactions. In VHL-deficient ccRCC tumors and cell lines, HIF1 α and HIF2 α may both be expressed, but HIF2 α is more consistently retained and functions as the dominant oncogenic driver. Genetic or pharmacological suppression of HIF2 α in VHL-deficient ccRCC models markedly inhibits tumor growth, highlighting its critical role in disease progression (49,53). Recognition of the central role of the VHL-HIF axis in ccRCC pathogenesis prompted the development of small-molecule inhibitors that selectively target HIF2 α . The discovery of a druggable cavity within the HIF2 α PAS-B domain enabled the development of selective antagonists such as PT2399 and PT2385, which bind HIF2 α allosterically and disrupt its heterodimerization with HIF1 β . Inhibition of HIF2 α

in preclinical studies reduced expression of downstream target genes, including *VEGFA* and *CCND1*, and produced substantial antitumor activity (100-102). Importantly, not all VHL-deficient tumors were sensitive to HIF2 α inhibition, providing early evidence of intrinsic HIF2 α -independent resistance (102).

PT2385 was subsequently evaluated in a phase I trial in patients with previously treated advanced ccRCC. The agent was generally well tolerated with no dose-limiting toxicities. Clinical activity included objective responses as well as prolonged disease stabilization in a subset of heavily pretreated patients (103). Nevertheless, in a molecular analysis of patients treated with PT2385, acquired HIF2 α G323E mutation impaired drug binding and preserved HIF2 α complex formation despite treatment (104). An additional consideration is the residual HIF1 α pool during selective HIF2 α inhibition. PT2385 dissociates HIF2 complexes and suppresses HIF2-dependent target genes while leaving HIF1 complexes largely unaffected (104). Because HIF1 α can exert tumor-suppressive effects in ccRCC, preservation of HIF1 signaling may be biologically advantageous rather than detrimental (52). Conversely, prolonged HIF2 α blockade may create selective pressure for HIF2-independent survival programs and resistance can emerge despite effective HIF2 inhibition (102,104). Direct evidence that HIF1 α itself mediates acquired resistance remains limited, whereas resistance through HIF2 α drug-binding mutations and HIF2-independent bypass pathways is better established. Recent clustered regularly interspaced short palindromic repeats-mediated transcriptional activation screening identified sustained *CCND1* expression as one such bypass mechanism, whereby maintenance of *CCND1* allowed ccRCC cells to proliferate despite HIF2 α inhibition (46). Whether restoration or preservation of HIF1 α activity could enhance the antitumor effects of selective HIF2 α inhibition remains elusive. HIF1 α may reinforce growth-restraining programs in certain VHL-deficient ccRCC contexts but direct evidence of additive or synergistic activity with HIF2 α inhibition is lacking. Given the context-dependent functions of HIF1 α , deliberate pharmacological activation of HIF1 α remains an unproven strategy that requires experimental validation (52,56,104).

PT2977 (also known as MK-6482 or belzutifan) is a second-generation HIF2 α inhibitor with improved pharmacological properties compared with PT2385 (105,106). A phase I trial demonstrated the safety and clinical activity of belzutifan in patients with previously treated advanced ccRCC (107). Belzutifan was subsequently evaluated in VHL disease-associated RCC, producing an objective response in 49% of patients in the pivotal phase II study (108). In 2021, belzutifan received Food and Drug Administration (FDA) approval for the treatment of adult patients with VHL disease who require therapy for associated RCC, central nervous system hemangioblastomas or pancreatic neuroendocrine tumors that do not require immediate surgery. Subsequently, the FDA extended belzutifan approval to patients with advanced RCC following prior PD-1/programmed death-ligand 1 (PD-L1) checkpoint inhibitor and VEGF-targeted TKI. This approval was based on the randomized phase III LITESPARK-005 trial. Although the median progression-free survival (PFS) was 5.6 months in both treatment groups, the overall PFS analysis significantly

favorable belzutifan over everolimus, with 24.0 vs. 8.3% of patients remaining alive and progression-free at 18 months. The objective response (ORR) rate was also substantially higher with belzutifan (21.9 vs. 3.5%) (109).

More recently, HIF2 α -directed therapy has expanded beyond previously treated advanced disease. In the phase III LITESPARK-022 trial, adjuvant belzutifan plus pembrolizumab significantly improved disease-free survival compared with pembrolizumab alone following nephrectomy in patients with ccRCC at increased risk of recurrence (hazard ratio, 0.72; 95% CI, 0.59-0.87), although OS data remain immature (110). These results led to FDA approval of the combination for adjuvant treatment, further extending the clinical relevance of HIF2 α inhibition across the ccRCC disease continuum. Collectively, these findings establish HIF2 α as a clinically validated therapeutic target in ccRCC. However, emerging resistance mechanisms highlight the need to better define HIF2-dependent tumors and the pathways that enable therapeutic escape.

VEGF/VEGFR-targeted antiangiogenic therapies. ccRCC is characterized by profound angiogenesis, largely driven by dysregulation of the VEGF/VEGFR signaling pathway. Among epithelial malignancies, ccRCC exhibits the highest levels of VEGFA expression, which provides a strong biological rationale for targeting VEGFA and its receptors, particularly VEGFR2 (111). The binding of VEGFA to VEGFR2 activates downstream signaling cascades that promote endothelial cell proliferation, migration and vascular permeability, making VEGFR2 the principal signaling receptor mediating angiogenesis in ccRCC (112). Within the TME, VEGFA is produced by ccRCC tumor cells as a consequence of VHL-HIF dysregulation and can also be produced by stromal and immune cells (57). This shift in favor of pro-angiogenic factors, commonly referred to as the 'angiogenic switch', is essential for tumor growth and progression (113).

Studies of anti-angiogenic therapy have focused mainly on the VEGFA-VEGFR signalling pathway. Early multi-targeted TKIs such as sorafenib, which inhibits VEGFR, PDGFR, FLT3 and BRAF, showed modest efficacy in advanced ccRCC, with an ORR of 10% and median PFS of 5.5 months vs. 2% and 2.8 months, respectively, for placebo, while median OS was 17.8 vs. 15.2 months (114). Direct evidence supporting VEGFA blockade came from a randomized trial in metastatic RCC, in which bevacizumab significantly delayed disease progression compared with placebo (115). Subsequent phase III studies demonstrated that bevacizumab combined with interferon (IFN)- α improved PFS compared with IFN- α alone, further validating VEGFA as a therapeutic target in RCC (116). In parallel, VEGFR-targeted TKIs emerged as an effective alternative strategy by inhibiting receptor tyrosine kinase activity and downstream angiogenic signaling. Sunitinib significantly improved PFS and the ORR compared with IFN- α in previously untreated metastatic RCC (117). Mechanistically, sunitinib suppresses VEGFR2 signaling in endothelial cells, reducing endothelial proliferation and migration as well as tumor microvessel density in ccRCC models (61).

Advances in ccRCC molecular biology have facilitated the development of multiple VEGFR-targeted TKIs, which are now central to the management of ccRCC. Sorafenib

was the first FDA-approved VEGFR inhibitor for advanced ccRCC, followed by sunitinib, which targets VEGFRs alongside PDGFRs, c-KIT, fms related receptor tyrosine kinase 3 and RET (117-119). Subsequent agents, including pazopanib, axitinib, lenvatinib and cabozantinib, exhibit broader or more selective kinase inhibition profiles and have demonstrated clinical efficacy in various treatment settings. Pazopanib significantly improved progression-free survival and objective response compared with placebo in advanced RCC (120). Notably, axitinib significantly prolonged PFS compared with sorafenib in advanced RCC (121), while cabozantinib improved ORR, PFS and OS compared with everolimus in patients previously treated with VEGFR-targeted therapy (122).

Despite the central role of VEGF/VEGFR-targeted therapies in the management of ccRCC, their clinical benefit is frequently limited by the emergence of adaptive resistance mechanisms. In response to sustained VEGF blockade, tumors can activate compensatory signaling pathways, alter metabolic programs and remodel the TME to promote growth and progression. For example, increased IL-8 signaling and activation of alternative receptor tyrosine kinases such as MET and AXL receptor tyrosine kinase have been implicated in resistance to sunitinib in ccRCC models (63,123). These findings illustrate the capacity of ccRCC to bypass sustained VEGF/VEGFR inhibition through alternative signaling programs, contributing to eventual treatment resistance.

mTOR pathway inhibition: Metabolic control and therapeutic limitations. Beyond angiogenic signaling, the PI3K-AKT-mTOR pathway represents another therapeutically relevant axis in ccRCC. Alterations affecting components of the PI3K/AKT/mTOR pathway occur in ~28% of ccRCC tumors, including alterations involving mTOR, PTEN and other pathway regulators (22). mTOR signaling can also be enhanced through mechanisms associated with VHL loss and HIF2 α activity, further linking this pathway to the molecular biology of ccRCC (71,75). mTOR inhibitors exert their antitumor effects primarily by disrupting mTORC1 signaling activity. mTORC1 normally promotes protein synthesis through phosphorylation of downstream effectors, particularly S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). Accordingly, inhibition of mTORC1 reduces S6K1 activity and 4E-BP1 phosphorylation, thereby restraining protein translation and cell proliferation (124). In 786-O and A498 ccRCC cell lines, mTOR inhibition induced G1-phase cell cycle arrest and suppressed tumor growth, providing preclinical support for the development of mTOR-targeted therapies in ccRCC (125).

This preclinical rationale translated into the clinical development of the rapamycin analogues everolimus and temsirolimus for advanced ccRCC. These mTOR inhibitors act as allosteric inhibitors by forming complexes with FK506-binding protein 12, which bind to mTOR within the mTORC1 complex and inhibit its kinase activity (124). However, these agents do not directly inhibit mTORC2, allowing AKT signaling to remain active or become reactivated through feedback mechanisms (126). Temsirolimus has been shown to improve OS compared with IFN- α in patients with poor-prognosis metastatic ccRCC (127). Similarly,

the RECORD-1 trial showed that everolimus significantly prolonged PFS compared with placebo in patients whose disease had progressed after VEGFR-targeted therapy (128).

Despite these benefits, responses to mTOR inhibitors are generally modest and therapeutic resistance commonly develops during treatment (129). Feedback activation of upstream PI3K-AKT signaling and incomplete inhibition of mTORC2 represent mechanisms that can limit the efficiency of mTORC1 blockade (126,129). With the emergence of more effective immune checkpoint inhibitor- and VEGFR-targeted regimens, the role of mTOR inhibitor monotherapy in advanced ccRCC has substantially diminished. Temsirolimus is now rarely used as first-line therapy, whereas everolimus retains a limited role in later-line treatment, particularly in combination with lenvatinib (130). This combination demonstrated greater clinical activity than everolimus alone in previously treated metastatic RCC (131). With the limited durability of mTOR-targeted monotherapy and increasing recognition of immune dysfunction in ccRCC, therapeutic strategies have increasingly shifted toward restoring antitumor immunity through immune checkpoint inhibition, either alone or in combination with targeted therapies (82).

Immune checkpoint blockade: Reshaping the immune-dysfunctional ccRCC microenvironment. Despite its high level of immune-cell infiltration, ccRCC is characterized by progressive immune dysfunction, including exhausted CD8⁺ T-cell populations and immunosuppressive myeloid states (82). These features provide a strong biological rationale for therapeutic strategies that restore antitumor immune activity within the TME. The immune checkpoint inhibitors target pathways that restrict antitumor immune responses. Among these, inhibitors of PD-1 and its ligand PD-L1 have gained particular prominence in ccRCC. PD-1 is expressed on activated and tumor-infiltrating T cells, whereas PD-L1 can be expressed by both tumor and immune cells within the ccRCC microenvironment (132,133). Their interaction suppresses T-cell activation and effector function, while blockade of the PD-1-PD-L1 axis can restore antitumor T-cell activity (134).

The clinical efficacy of PD-1 blockade in advanced ccRCC was established by the phase III CheckMate 025 trial, in which nivolumab significantly improved median OS compared with everolimus in previously treated patients (135). Long-term follow-up confirmed sustained survival and response benefits, demonstrating that a subset of patients can achieve prolonged disease control with PD-1 inhibition (136). The activity of PD-1 blockade was also demonstrated in the first-line setting in KEYNOTE-427, where pembrolizumab monotherapy achieved an objective response rate of 36.4% in treatment-naïve patients with advanced ccRCC (137). Furthermore, pembrolizumab has also demonstrated benefit in the adjuvant setting. In the phase III KEYNOTE-564 trial, pembrolizumab given after nephrectomy improved disease-free survival and OS in patients with ccRCC at increased risk of recurrence (138).

Another major immune checkpoint is cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which regulates T-cell activation through a mechanism distinct from PD-1. CTLA-4 competes with the co-stimulatory receptor CD28 for binding to CD80 and CD86 on antigen-presenting cells, hence limiting T-cell activation and expansion (139). Early studies

of the CTLA-4 inhibitor ipilimumab demonstrated antitumor activity in a subset of patients with metastatic RCC, although responses were modest and accompanied by substantial immune-related toxicity (140). The complementary functions of CTLA-4 and PD-1 subsequently provided the rationale for dual checkpoint blockade. In the phase III CheckMate 214 trial, nivolumab plus ipilimumab produced durable responses and superior OS compared with sunitinib in patients with advanced RCC. Long-term follow-up confirmed that these survival and response benefits were sustained over time (141). These findings established combined PD-1 and CTLA-4 blockade as an important first-line treatment strategy for advanced ccRCC. Nevertheless, primary and acquired resistance remain important limitations and durable responses are achieved in only a subset of patients (142). These limitations have stimulated investigation of additional immune checkpoints and rational combination strategies. The integration of checkpoint blockade with VEGFR-targeted therapy represents one of the most clinically successful examples and is discussed in the following subsection.

Combination strategies: Integrating angiogenic, immune and metabolic vulnerabilities. The therapeutic landscape of ccRCC has progressively shifted towards combination treatment strategies aimed at improving antitumor efficacy by targeting complementary biological vulnerabilities. The rationale for combining VEGF/VEGFR inhibition with immune checkpoint blockade is particularly strong in ccRCC. As discussed in the preceding section, abnormal angiogenesis and immune dysfunction are closely interconnected. In metastatic ccRCC, bevacizumab combined with PD-L1 blockade was shown to increase intratumoral CD8⁺ T-cell infiltration and antigen-specific T-cell migration, providing direct evidence for this vascular-immune interaction (143). VEGFR TKIs can further complement checkpoint blockade through their effects on angiogenic and tumor-cell signaling. Axitinib predominantly targets VEGFR1-3, whereas cabozantinib additionally inhibits kinases including MET and AXL, and lenvatinib targets VEGFRs together with other receptor tyrosine kinases (144). These broader activities may help suppress alternative signaling pathways that contribute to tumor progression and resistance to VEGF blockade (57).

The clinical efficacy of ICI-TKI combinations has been established in several phase III trials. In KEYNOTE-426, pembrolizumab plus axitinib improved OS and PFS compared with sunitinib, with sustained benefit after >5 years of follow-up (145). Similarly, nivolumab plus cabozantinib demonstrated sustained improvements in survival and response outcomes over sunitinib in CheckMate 9ER, with final long-term analysis confirming its benefit as first-line therapy (146). In the CLEAR trial, lenvatinib plus pembrolizumab also produced significant and durable improvements in PFS and OS compared with sunitinib (147). Avelumab, a PD-L1 inhibitor, combined with axitinib, improved PFS and the ORR in the JAVELIN Renal 101 trial, although the final OS analysis did not demonstrate a statistically significant benefit (148). Furthermore, combination strategies have also extended beyond immune-angiogenic therapy. As discussed above, lenvatinib combined with

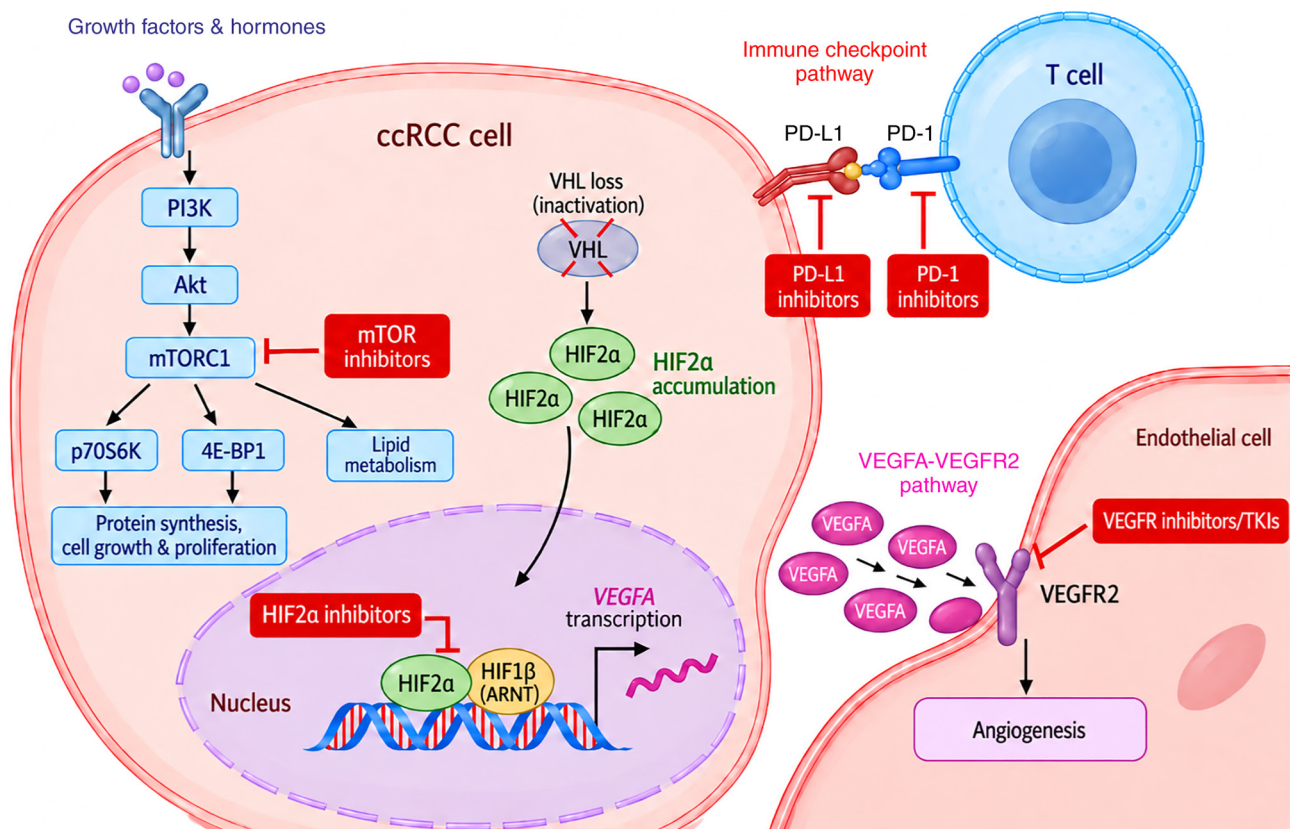


Figure 3. Major oncogenic pathways and therapeutic targets in ccRCC. VHL loss promotes HIF2 α accumulation and VEGFA transcription, driving angiogenesis through VEGFR2 signaling. PI3K/AKT/mTORC1 signaling supports cell growth, proliferation and lipid metabolism while PD-1/PD-L1 signaling contributes to immune evasion. Major therapeutic targets include HIF2 α , VEGFR, mTOR and immune checkpoints. HIF, hypoxia-inducible factor; HIF2 α , hypoxia-inducible factor 2 alpha; HIF1 β , hypoxia-inducible factor 1 beta; ARNT, aryl hydrocarbon receptor nuclear translocator; ccRCC, clear cell renal cell carcinoma; VHL, von Hippel-Lindau; VEGFA, vascular endothelial growth factor A; VEGFR, vascular endothelial growth factor receptor; VEGFR2, vascular endothelial growth factor receptor 2; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; p70S6K, 70-kDa ribosomal protein S6 kinase; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; TKI, tyrosine kinase inhibitor.

everolimus demonstrated greater activity than everolimus alone (131). This provides an example of simultaneous targeting of angiogenic and mTOR-dependent growth pathways. Together, these clinical advances have established ICI-TKI combinations, dual checkpoint blockade and selected TKI-mTOR regimens as important components of systemic therapy for advanced ccRCC. The major oncogenic pathways and corresponding therapeutic targets discussed in this section are summarized in Fig. 3. Several of the FDA-approved therapies and their key clinical efficacy outcomes are summarized in Table I (65,66,86, 109,114,120,122,127,128,131,149-152).

9. Future directions: Toward precision and microenvironment-informed therapy

Significant advances in ccRCC treatment have shifted management from non-specific approaches toward targeted and immune-based therapies. Nevertheless, tumor recurrence and therapeutic resistance remain major challenges due to extensive genetic, epigenetic and microenvironmental heterogeneity of ccRCC. Therefore, future progress will depend not only on the development of new therapeutic agents but also on identifying biomarkers that more accurately capture tumor

dependencies and treatment response, enabling more precise patient stratification and therapeutic selection.

Single-cell and spatial profiling provide robust platforms for resolving distinct malignant, immune and stromal states within the ccRCC microenvironment that may influence disease progression and therapeutic response. Multi-omics studies have further demonstrated substantial intratumoral variation in molecular, immune and metabolic programs, as well as spatially distinct metabolic states associated with ccRCC progression (78,80). Integration of these datasets with computational and machine-learning approaches may facilitate the development of predictive models for treatment response and support precision medicine in ccRCC. To this end, spatial metabolomics provides a complementary approach by preserving tissue architecture while mapping regional metabolic programs that will potentially reveal the metabolically distinct tumor niches that are not captured by bulk profiling (80,153).

Beyond molecular profiling, emerging nanomedicine strategies provide opportunities to therapeutically modify features of the hypoxic TME. Wang *et al* (154) developed oxygen-carrying semiconducting polymer nanopropdrugs to relieve tumor hypoxia. The system enhanced ultrasound-triggered reactive oxygen species (ROS) generation, induced

Table I. Selected FDA-approved drugs and combination therapies for advanced ccRCC.

Drug class	Compound (FDA approval year)	Target(s)	Number of patients/phase of trial	Effects on ccRCC or efficacy			Side effects	(Refs.)
				ORR	PFS (median in months)	Median OS, months		
HIF2 α inhibitor Antiangiogenic VEGF/VEGFR inhibitors (mAbs + TKIs)	Belzutifan (2023)	HIF2 α	746/III	Belzutifan 21.9% vs. everolimus 3.5%	Belzutifan 5.6 vs. everolimus 5.6	Belzutifan 21.4 vs. everolimus 18.1	Hypoxia, anaemia and fatigue	(109)
	Lenvatinib + everolimus (2016)	VEGFR, FGFR, mTORC1	153/II	Lenvatinib + everolimus 43% vs. everolimus 6%	Lenvatinib + everolimus 14.6 vs. everolimus 5.5	Lenvatinib + everolimus 25.5 vs. everolimus 15.4	Diarrhoea, fatigue and hypertension	(131)
	Cabozantinib (2016)	VEGFR, MET, AXL	658/III	Cabozantinib 17% vs. everolimus 3%	Cabozantinib 7.4 vs. everolimus 3.8	Cabozantinib 21.4 vs. everolimus 16.5	Diarrhoea, palmar-plantar erythrodysesthesia syndrome, fatigue and hypertension	(122)
	Axitinib (2012)	VEGFR, PDGFR, c-KIT	723/III	Axitinib 19% vs. sorafenib 9%	Axitinib 8.3 vs. sorafenib 5.7	Axitinib 20.1 vs. sorafenib 19.2	Hypertension, diarrhoea, fatigue and hand-foot syndrome	(149)
	Pazopanib (2009)	VEGFR, PDGFR, c-KIT, FGFR	435/III	Pazopanib 30% vs. placebo 3%	Pazopanib 9.2 vs. placebo 4.2	NR vs. NR	Diarrhoea, hypertension, nausea and vomiting	(120)
mTOR inhibitor	Bevacizumab + IFN- α (2009)	VEGFA	649/III	Bevacizumab + IFN- α 31% vs. IFN- α 13%	Bevacizumab + IFN- α 10.2 vs. IFN- α 5.4	Bevacizumab + IFN- α 23.3 vs. IFN- α 21.3	Hypertension, anorexia, fatigue and proteinuria	(150)
	Sunitinib (2006)	VEGFR, PDGFR, c-KIT	750/III	Sunitinib 47% vs. IFN- α 12%	Sunitinib 11 vs. IFN- α 5	Sunitinib 26.4 vs. IFN- α 21.8	Hypertension, fatigue, diarrhoea and hand-foot syndrome	(151)
	Sorafenib (2005)	VEGFR, PDGFR, FLT3, BRAF	903/III	Sorafenib 10% vs. placebo 2%	Sorafenib 5.5 vs. placebo 2.8	Sorafenib 17.8 vs. placebo 15.2	Diarrhoea, fatigue, hypertension, hand-foot skin reaction	(114)
	Everolimus (2009)	mTORC1	416/III	Everolimus 2% vs. placebo 0%	Everolimus 5.5 vs. placebo 1.9	Everolimus 14.8 vs. placebo 14.4	Infection (all types), dyspnoea and fatigue	(128)
	Temsirolimus (2007)	mTORC1	626/III	Temsirolimus 8.6% vs. IFN- α 4.8% vs. temsirolimus + IFN- α 8.1%	Temsirolimus 3.8 vs. IFN- α 1.9 vs. temsirolimus + IFN- α 3.7	Temsirolimus 10.9 vs. IFN- α 7.3 vs. temsirolimus + IFN- α 8.4	Dyspnoea, diarrhoea, nausea and vomiting	(127)

Table I. Continued.

Drug class	Compound (FDA approval year)	Target(s)	Number of patients/phase of trial	Effects on ccRCC or efficacy				Side effects	(Refs.)
				ORR	PFS (median in months)	Median OS in months			
Immuno-therapies (immune checkpoint inhibitors)	Pembrolizumab + axitinib (2019)	PD-1, VEGFR	861/III	Pembrolizumab + axitinib 59.3% vs. sunitinib 35.7%	Pembrolizumab + axitinib 15.1 vs. sunitinib 11.1	NR vs. NR		Diarrhoea and hypertension	(65)
	Avelumab + axitinib (2019)	PD-L1, VEGFR	886/III	Avelumab + axitinib 51.4% vs. sunitinib 25.7%	Avelumab + axitinib 13.8 vs. sunitinib 8.4	NR vs. NR		Diarrhoea, hypertension and fatigue	(86)
	Nivolumab + cabozantinib (2021)	PD-1, VEGFR, MET, AXL	651/III	Nivolumab + cabozantinib 55.7% vs. sunitinib 27.1%	Nivolumab + cabozantinib 16.6 vs. sunitinib 8.3	NR vs. NR		Palmar-plantar erythrodysesthesia, hypertension, hypothyroidism and fatigue	(66)
	Nivolumab + ipilimumab ^a (2018)	PD-1, CTLA-4	1096/III	Nivolumab + ipilimumab 42% vs. sunitinib 27%	Nivolumab + ipilimumab 11.6 vs. sunitinib 8.4	Nivolumab + ipilimumab NR vs. sunitinib 26.0		Fatigue, diarrhoea, rash, and increased lipase/amylase	(152)

^aEfficacy outcomes are shown for patients with intermediate/poor IMDC risk. n, number of samples; FDA, Food and Drug Administration; ccRCC, clear cell renal cell carcinoma; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; mAbs, monoclonal antibodies; HIF2 α , hypoxia-inducible factor 2 α ; VEGFA, vascular endothelial growth factor A; VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptors; MET, mesenchymal-epithelial transition factor; AXL, AXL receptor tyrosine kinase; PDGFR, platelet-derived growth factor receptor; FLT3, Fms-like tyrosine kinase 3; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; IFN- α , interferon α ; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium; NR, not reported.

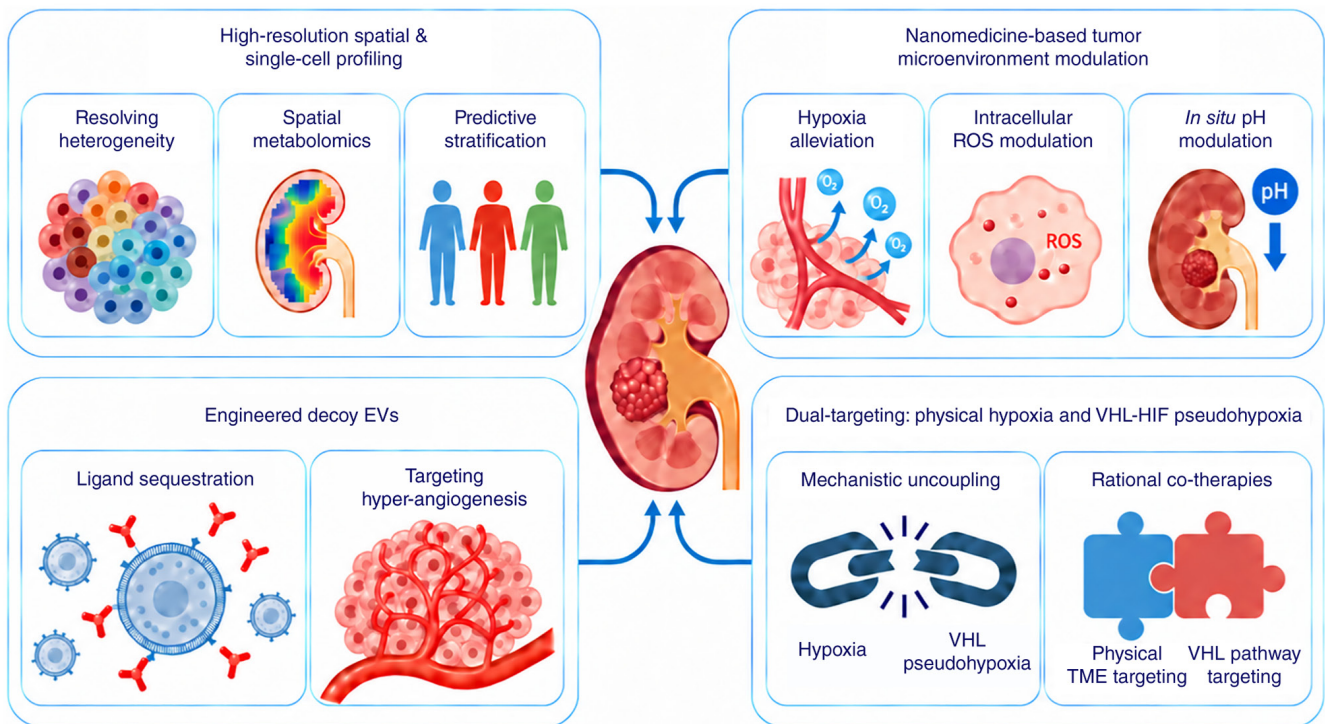


Figure 4. Emerging precision or complementary therapeutic strategies for ccRCC. These approaches include high-resolution spatial and single-cell profiling, nanomedicine-based modulation of the TME, engineered decoy extracellular vesicles and dual targeting of physical hypoxia and VHL-HIF-driven pseudohypoxia. HIF, hypoxia-inducible factor; ccRCC, clear cell renal cell carcinoma; VHL, von Hippel-Lindau; ROS, reactive oxygen species; TME, tumor microenvironment.

pyroptosis and increased antitumor immune-cell infiltration, leading to reduced tumor growth and metastasis (154). Tang *et al* (155) developed pH-responsive artesunate-based nanoparticles. These nanoparticles increased mitochondrial heme, ROS production and lipid peroxidation, thereby improving combined chemotherapy and chemodynamic therapy. Chen *et al* (156) designed hemoporphin-loaded liposomes containing catalase and L-buthionine sulfoximine. This platform increased intratumoral oxygen generation and reduced glutathione-mediated ROS scavenging, which enhanced photodynamic therapy (156). Deng *et al* (157) combined hyperbaric oxygen with acid-responsive copper-doped hydroxyethyl starch nanoparticles. This approach promoted intracellular acidification, drug release and copper-mediated chemodynamic activity against tumor and cancer stem-like cells (157). Although these approaches have not yet been established in ccRCC, they illustrate how hypoxia, redox balance and other properties of the TME may be therapeutically exploited.

However, translating these hypoxia-directed strategies to ccRCC requires consideration of the distinction between true hypoxia and VHL-driven pseudohypoxia. In VHL-deficient ccRCC, HIF2 α signaling remains constitutively active even when oxygen availability is adequate (19). Consequently, increasing tumor oxygenation alone would not be expected to suppress the oncogenic VHL-HIF2 α program. Microenvironment-directed approaches may therefore be more effective when combined with direct targeting of HIF2 α , angiogenic signaling, epigenetic dependencies or immune checkpoints. This distinction highlights the need to consider both the physical hypoxic

microenvironment and the genetically encoded pseudohypoxic state when developing new therapeutic strategies for ccRCC.

Engineered extracellular vesicles (EVs) represent another emerging strategy for modulating TME signaling. EVs can be modified to display functional proteins or decoy receptors on their surface, which enable them to bind and sequester soluble ligands before receptor activation on target cells. Proof-of-concept studies have demonstrated that engineered EVs displaying cytokine-binding receptors can neutralize extracellular ligands and attenuate downstream signaling (158,159). In highly angiogenic tumors such as ccRCC, this concept could potentially be adapted to display receptors capable of sequestering pro-angiogenic ligands such as VEGFA, thereby reducing their availability within the TME. Such receptor-engineered EVs could complement existing anti-angiogenic strategies by intercepting extracellular signaling before activation of tumor- or stromal-cell receptors. However, their ligand-capture efficiency, biodistribution, safety and therapeutic efficacy in ccRCC remain to be established.

A key next step will be to move from descriptive molecular profiling toward functional validation of spatially resolved tumor states. Integrating patient-derived models and perturbation-based functional genomics with longitudinal sampling before and after therapy could help identify mechanisms that drive tumor progression and treatment resistance. A deeper understanding of how genetic, epigenetic and microenvironmental features interact to sustain pseudohypoxic signaling and therapeutic resistance may enable more precise and durable treatment strategies. These

emerging precision or complementary therapeutic strategies are summarized in Fig. 4.

10. Conclusion

ccRCC can be viewed as a malignancy organized around a persistent 'false sense of hypoxia'. Loss of VHL uncouples oxygen sensing from HIF degradation, allowing HIF2 α -dependent transcription to remain active under normoxic conditions and thereby sustaining angiogenesis, metabolic reprogramming, immune dysfunction and tumor-cell adaptation. This pseudohypoxic state is further reinforced by intratumor heterogeneity and epigenetic reprogramming, including super-enhancer-driven transcriptional dependencies that support growth and metastatic progression. The clinical success of VEGF/VEGFR-directed therapies, immune checkpoint blockade and selective HIF2 α inhibition validates key components of this biological framework. However, durable disease control remains limited by adaptive and acquired resistance. Future progress will therefore require a better understanding of the molecular, epigenetic, spatial and microenvironmental determinants of therapeutic response and resistance. In this context, rational combination strategies should aim not only to suppress individual pathways but also to disrupt the interconnected pseudohypoxic networks that enable ccRCC cells to adapt and persist under therapeutic pressure.

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Availability of data and materials

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Authors' contribution

NQAAR and SES wrote the manuscript. ASK and MAM performed the literature search and revised the manuscript. NQAAR and ASK generated the figures and table. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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