Clinical Cancer Research

New Strategies in Renal Cell Carcinoma: Targeting the Genetic and Metabolic Basis of Disease

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Abstract

The development of new forms of treatment of advanced renal cell carcinoma over the past two decades has been primarily focused on targeting the VHL/HIF pathway. The recent identification of mutations of chromatin-remodeling genes in clear-cell renal carcinoma (ccRCC), of genomic heterogeneity, and of a Warburg-like metabolic phenotype in advanced disease has had a profound effect on our understanding of the evolution of ccRCC and on potential approaches to personalized therapy. Early approaches to therapy for patients with advanced type I papillary RCC that have centered around the MET/HGF pathway will expand as more genomic information becomes available. Sporadic and familial type II papillary renal cell carcinoma are characterized by enhanced aerobic glycolysis and share an antioxidant response phenotype. In fumarate hydratase–deficient RCC, fumarate-induced succination of KEAP1 activates Nrf2 signaling. CUL3 and Nrf2 mutations as well as an Nrf2 activation phenotype are found in sporadic type II papillary RCC. Therapeutic approaches designed to target the Nrf2 pathway as well as to impair blood flow and glucose delivery in these cancers that are highly dependent on a robust tumor vasculature and on ready availability of glucose for energy production and glycolysis are in development. *Clin Cancer Res; 21(1); 10–17. ©2015 AACR.*

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Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the strategies currently in use and under investigation for therapy for renal cell carcinoma and of the biologic rationale underlying novel therapeutic strategies.

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Background

Renal cell carcinoma (RCC) affects more than 270,000 individuals annually worldwide, and there are nearly 120,000 deaths each year due to this disease (1). Patients with relatively small, localized (T1) disease treated surgically have excellent 5- and 10-year survival rates (95%). However, in patients with advanced disease (T4 or M1), the 2-year survival was less than 20% before the advent of targeted agents directed against the HIF/VEGF pathway. Our ability to better manage patients with advanced disease has been largely driven by a better understanding of the genetic and biochemical alterations underlying these tumors. Over the past two decades, it has become clear that RCC is not a single disease but is made up of a number of different types of cancer that occur in this organ, each with a different histology, a different clinical course, responding differently to therapy, and caused by different genes. The morphologic classification of RCC is on its way to becoming obsolete as we discover the genomic and molecular drivers of these diseases. Much of what has been learned about the genetic basis of RCC has come from study of the inherited forms of RCC, including von Hippel–Lindau (clear-cell RCC), hereditary papillary renal carcinoma (type I papillary RCC), and hereditary leiomyomatosis RCC (type II papillary RCC; refs. 2, 3). Over the past decade, significant progress has been made in the development of targeted therapeutic approaches for patients with advanced RCC (4). However, despite the advent of these new

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agents, most patients with advanced RCC progress on therapy and many will eventually die of this disease.

Clear-cell RCC (ccRCC) is the most common type of RCC and accounts for 75% of cases. The *VHL* tumor suppressor gene, located on the short arm of chromosome 3, is the gene for the inherited form of ccRCC associated with von Hippel–Lindau (5) and has been found to be mutated or methylated in a high percentage of tumors (~90%) from patients with ccRCC (6, 7). The VHL protein forms a complex with elongin C, elongin B, and CUL2 to target the hypoxia-inducible factors, HIF1α and HIF2α for ubiquitin-mediated degradation (8–10). This is an oxygen-sensing process; in normoxia, HIF prolyl hydroxylase (PHD) transfers hydroxyl groups to two prolines in HIF, which enables the VHL complex to target and degrade HIF. In hypoxia, PHD does not hydroxylate HIF, the VHL complex cannot target and degrade HIF, and HIF accumulates. HIF1α and HIF2α are transcription factors that regulate the transcription of a number of hypoxia-responsive genes, such as VEGF, platelet-derived growth factor, and glucose transporters GLUT1 and GLUT4. Understanding the VHL/HIF pathway has provided the foundation for the development of a number of targeted therapeutic approaches for the treatment of patients with advanced ccRCC, leading to the development and FDA approval of several agents that target this pathway. Temsirolimus and everolimus, which target the mTOR pathway, have also been approved for advanced RCC. While these agents have had remarkable clinical effect, up to a 45% response rate and increased progression-free survival rates, virtually all eventually develop progressive disease (11).

Papillary RCC, which accounts for 15% of RCC, is made up of a heterogeneous group of cancers that encompasses multiple histologic subtypes, including type I papillary RCC and type II papillary RCC. Although inhibition of the VEGF or mTOR signaling pathways has led to some clinical benefits, the effect is marginal and the prognosis remains poor for patients with advanced disease (12).

On the Horizon

Clear cell renall cell carcinoma

Targeting the VHL/HIF pathway

Why is targeting the VHL/HIF pathway in ccRCC only partially successful clinically? Is it because we need to develop more effective ways to target this pathway or because we need to identify and target other genes associated with the development of this disease. The use of more target-specific, potent VEGFR inhibitors has not been shown to improve efficacy but may be associated with a better toxicity profile. Concurrent inhibition of VEGFR and MET, Tie2, FGFR, and mTOR pathways are being evaluated; however, complete remissions and long-term responses are rare with these strategies (11). Functional studies have long suggested that HIF2α (versus HIF1α) is the critical downstream target of VHL (13, 14) and that inhibition of HIF2α can suppress the ability of a $VHL^{-/-}$ tumor to form tumors in xenograft models (15, 16) and HIF2α SNPs have been linked to an increased risk of developing RCC (17, 18). Genetic and functional studies have implicated HIF1α as a chromosome 14q kidney cancer suppressor gene (19) and chromosome 14q loss has been found to be associated with poor prognosis (20). A number of strategies have been developed to target HIF2α. The Molecular Targets Laboratory at the National Cancer Institute (Bethesda, MD) has isolated a number of agents that have been shown to inhibit HIF2α transcriptional activity that will need to be further studied in *in vitro* and *in vivo* models to determine specificity and effectiveness (21–24). Zimmer and colleagues have reported small-molecule inhibitors of HIF2α translation based on enhancing the binding activity of IRP1 to the iron responsive element (IRE) in the 5′-untranslated region of the HIF2α message (25, 26). Utilizing a similar approach, tempol, a stable nitroxide that activates IRP1 toward IRE binding, has been shown to decrease HIF2α and its downstream targets in $VHL^{-/-}$ RCC cell line models (27). The role of this agent alone or in combination with other antitumor agents will need to be evaluated in *in vitro* and *in vivo* models. Additional approaches involving autophagic degradation of HIF2α (28), targeting proteosomal degradation of VHL missense mutations (29), and targeting the hypoxia-associated factor–mediated regulation of HIF2α-dependent transcription during hypoxia are being evaluated (30, 31).

Chromatin-remodeling gene mutations

Dalgliesh and colleagues opened a new chapter in our understanding of the genetic basis of ccRCC and the role of systematic screens to determine genomic architecture when they reported the identification of inactivating mutations of genes involved in histone modification, such as the histone H3 lysine 36 methyltransferase *SETD2* and the histone H3 lysine 4 demethylase *KDM5C* (32). This work was followed shortly by the identification of truncating mutations of the SWI/SNF chromatin-remodeling complex gene, *PBRM1*, and BAP1, a deubiquitase that is the catalytic subunit of the Polycomb repressive deubiquitinase complex, in ccRCC, highlighting the fundamental role of aberrant chromatin biology in this disease (33, 34). The Cancer Genome Atlas (TCGA) performed a comprehensive molecular characterization of more than 400 ccRCCs and identified 19 significantly mutated genes, including *VHL* and other chromosome 3 genes such as *PBRM1*, *SETD2*, and *BAP1*, as well as *KDM5C*, *PTEN*, *MTOR*, *TP53*, and *PIK3CA*. Mutations in the chromatin regulators, *SETD2*, *BAP1*, and *PBRM1* were each differentially associated with altered expression patterns with a distinct set of downstream effects, reflecting different roles for chromatin remodeling in the transcriptome (35). A number of findings have highlighted the importance of driver mutations of chromatin-remodeling genes in ccRCC. In large studies, somatic mutations of *BAP1* have been correlated with decreased overall survival, and a straightforward immunohistochemical assay has been developed to assess BAP1 levels (7, 35–38). In addition, germline BAP1 mutations have been found to be the driver mutations in families characterized by an increased incidence of early onset, bilateral, multifocal ccRCC (39, 40). Intensive efforts are currently under way to determine the functional consequences of inactivation of chromatin remodeling genes in ccRCC to provide the foundation for the development of effective forms of therapy targeting these gene pathways. Simon and colleagues have recently identified alterations in chromatin organization and transcript profiles in ccRCC associated with SETD2 mutations in a large cohort of primary human kidney tumors (41) as a first step in developing actionable targets in SETD2-deficient ccRCC. The findings that SETD2 has been shown to be required for DNA double-strand break repair and activation of the p53-mediated checkpoint (42), that SETD2-dependent histone H3K36 trimethylation is required for homologous recombination repair and genome stability (43), and that cells lacking SETD2 activity display microsatellite instability and an elevated spontaneous

mutation frequency characteristic of DNA mismatch repair–deficient cells (44) opens the door to therapeutic approaches targeting genomic instability in SETD2-deficient RCCs.

Genomic heterogeneity in ccRCC

In studies that have profound implications for both understanding ccRCC tumor evolution as well as for the development of effective strategies for therapy of this disease, Gerlinger and colleagues performed multiregion exome sequencing (M-seq) in large tumors from 10 patients (all except two of whom presented with metastatic disease) and identified pronounced intratumoral heterogeneity (45, 46). *VHL* gene mutation, which was found in each sample, was considered to be a "truncal" mutation as it was uniformly present in all segments. *PBRM1* was found to be mutated in a portion of the tumors and was also considered to be truncal in those subsets. Other driver mutations, found in the chromatin-remodeling genes *BAP1*, *KDM5C*, and *SETD2*, genes in the phosphoinositide 3-kinase (PI3K)–mTOR pathway (*mTOR*, *PIK3CA*, *TSC2*, *PTEN*) and *TP53*, were found only in "branch" segments of the tumors and each portion of the tumors was found to have a unique spectrum of branch mutations. As more portions of the tumors were samples, more tumor heterogeneity was identified, suggesting that even these studies underestimate the true extent of heterogeneity of driver mutations in ccRCC (45–47).

These studies raise a number of very fundamental questions. First, what is the best way to detect driver mutations in ccRCC? One approach could involve performing M-seq on the primary kidney tumor to identify druggable driver mutations for therapy. Another approach could involve performing M-seq on tumor material from metastatic tumors, considering that the tumor that has already metastasized might be most likely to contain the critical driver mutations. The second and more critical question is how should we develop a therapy for a tumor with such genomic heterogeneity. One approach would be to continue to focus on developing more effective approaches to targeting the primary truncal mutation (the VHL pathway). When more information about the effect of mutations in chromatin-remodeling genes such as *SETD2* and *BAP1* becomes available, approaches targeting these pathways will be evaluated.

Targeting the metabolic basis of ccRCC

Integrative analysis in the TCGA study revealed that high-grade, high-stage ccRCC from patients with poor survival showed evidence of a metabolic shift, involving downregulation of genes involved in the TCA cycle, decreased PTEN and AMPK protein levels, upregulation of the glutamine transporter and pentose phosphate pathway genes, and increased acetyl-CoA carboxylase protein (35). The finding of a metabolic shift involving dependence on the pentose phosphate shunt, decreased AMPK, increased glutamine transport, and fatty acid production is consistent with the findings of VHL-deficient ccRCC lines that preferentially use glutamine-dependent reductive carboxylation for lipid biosynthesis (48), which is regulated by citrate levels and sensitizes VHL-deficient cells to glutamine deprivation (49). The balance between reductive carboxylation and glucose catabolism in VHL-deficient cells is coordinated by nicotinamide transhydrogenase (50). The finding of a Warburg shift in ccRCC, consistent with that seen in fumarate hydratase (FH)-deficient RCC (51), provides a number of opportunities for targeted therapies (Fig. 1). A number of potential targets are available, including glutaminase, AMPK, FAS, and LDHA. Agents targeting AMPK, such as metformin and AICAR, have shown antitumor activity in *in vitro* RCC cell line models (52). Glutaminase inhibitors have shown efficacy in VHL-deficient RCC cell line models (49) and are currently being evaluated in clinical trials. Intense efforts are under way to identify an effective LDHA inhibitor appropriate for clinical use.

Papillary RCC

Type I papillary RCC

Type I papillary RCC occurs in a sporadic, noninherited as well as a hereditary form. Hereditary papillary kidney (HPRC) cancer is an autosomal dominant hereditary cancer syndrome in which affected individuals are at risk for the development bilateral, multifocal type I papillary RCC (53). The gene for HPRC, localized to the long arm of chromosome 7, was found to be the proto-oncogene *MET* (54). Activating mutations in the tyrosine kinase domain of *MET* are found in the germline of patients affected with HPRC (54, 55). Kidney tumors from patients affected with both HPRC and sporadic papillary RCC are characterized by polysomy of chromosome 7, and nonrandom duplication of the mutant *MET* allele has been found in tumors from patients affected with HPRC (56). Hereditary and sporadic type I papillary RCC share a distinct morphologic phenotype (57), and *MET* mutations have been found in 13% of sporadic type I papillary tumors.

The identification of oncogenic *MET* mutations in patients affected with HPRC and in a subset of patients with sporadic papillary RCC led to a multicenter phase II study of foretinib, an inhibitor of Met, VEGFR2, RON, and AXL tyrosine kinases, in patients with sporadic as well as HPRC-associated papillary RCC. The overall response rate was 13.5%, with a median progression-free survival of 9.3 months, which was considerably higher than that seen in historical controls treated with agents targeting mTOR or VEGFR pathways (58). In a subgroup analysis, 5 of 10 (50%) patients with HPRC with germline *MET* mutations were found to have a partial response to therapy, versus 5 of 57 (9%) non-HPRC patients. Sufficient pathology review was not available to determine whether type I papillary RCC responded better than type II papillary RCC (58). A clinical trial evaluating the effect of INC280, an agent that selectively targets Met, in patients with papillary RCC is under way. Trials are currently being planned to evaluate the effect of a number of agents that target Met in patients with both papillary and nonpapillary RCC. The TCGA papillary RCC project is currently under way to provide more insights into the genetic characteristics of both type I and type II papillary RCC and will hopefully identify driver mutations that will guide future trials in this disease.

Type II papillary RCC

Type II papillary kidney cancer is an aggressive and often lethal form of RCC (2, 3). As is the case with type I papillary RCC, there are both sporadic as well as familial forms of type II papillary RCC. The hereditary form of type II papillary RCC occurs in hereditary leiomyomatosis and RCC (HLRCC). HLRCC is an autosomal dominant hereditary cancer syndrome in which patients are at risk for the development of cutaneous and uterine leiomyomas and a very aggressive, lethal form of type II papillary RCC that has a propensity to spread when the primary tumor is very small

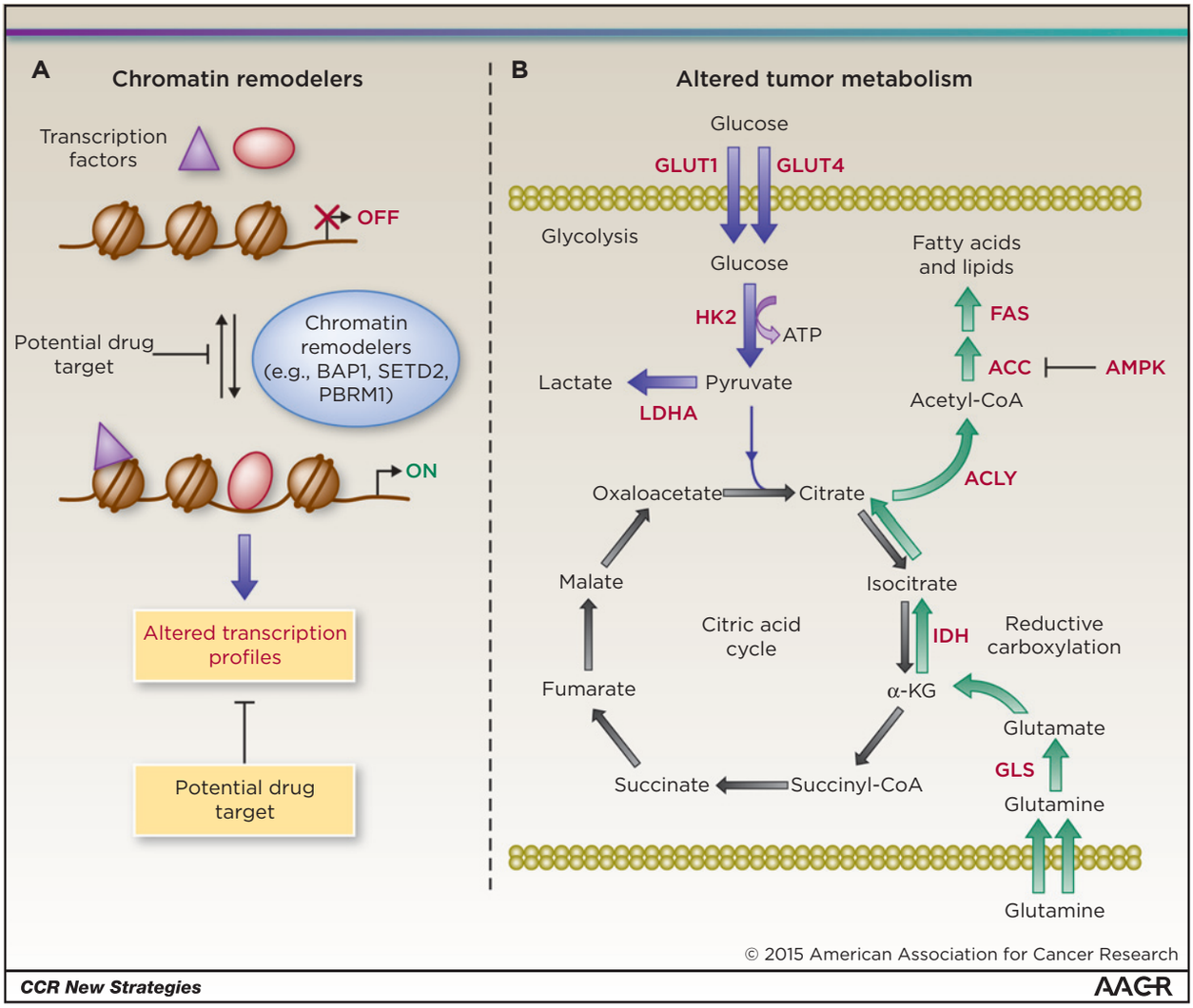

Figure 1.
Potential therapeutic targets for ccRCC. Potentially drug targetable pathways associated with ccRCC include chromatin remodeling and altered tumor metabolism. A, mutations in several chromatin-remodeling genes such as *PBRM1*, *SETD2*, and *BAP1* are common in ccRCC and result in altered transcription profiles due to the specific effects of loss of each of these genes. As knowledge of these pathways increases, potential therapies could target downstream transcriptional gain-of-function or loss-of-function events. B, higher grade, high-stage low survival ccRCCs are associated with a metabolic shift consistent with a suppression of oxidative phosphorylation and a subsequent dependence upon glycolysis for energy. This results in a dependence on glucose for ATP and increased conversion of pyruvate to lactate for excretion while suppression of AMP-activated protein kinase (AMPK) aids fatty acid production and growth. The enzymes and transporters involved in these processes, such as Hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), and glucose transporters 1/4 (GLUT1/4), are potential targets (highlighted in red). In addition, the increased need for fatty acids for growth requires an alternative carbon metabolite to enter the citric acid cycle to produce the necessary citrate. These tumors would increase their glutamine uptake and conversion to glutamate for entry into the citric acid cycle and both the glutaminase (GLS) and fatty acid synthesis enzymes, such as ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS), are potential therapeutic targets. Because of the suppression of the normal flow in the citric acid cycle, the alternative reductive carboxylation pathway that is used to convert α-ketoglutarate (α-KG) to citrate via the isocitrate dehydrogenase (IDH) enzymes provides further potential targets.

(59–61). HLRCC is characterized by germline mutation of the gene for the Krebs cycle enzyme, FH (62).

Warburg model of cancer

In FH-deficient RCC, oxidative phosphorylation is impaired and the cells undergo a shift to aerobic glycolysis, consistent with the Warburg effect (63, 64). FH-deficient RCC cells are unusually dependent on glucose for proliferation and glycolysis to generate the increased ATP needed for rapid proliferation (65). In FH-deficient RCC, fumarate accumulates and inhibits HIF PHD, resulting in accumulation of HIF1 and increased transcription of its targets, such as VEGF and GLUT1, which leads to the increased vascularity and glucose transport needed to provide increased nutrients for rapid growth and proliferation and for ATP production needed in cells characterized by a decrease in oxidative phosphorylation (Fig. 2; ref. 66). Diminished AMPK levels have been found in FH-deficient RCC cells, resulting in reduced phosphoryhlation of

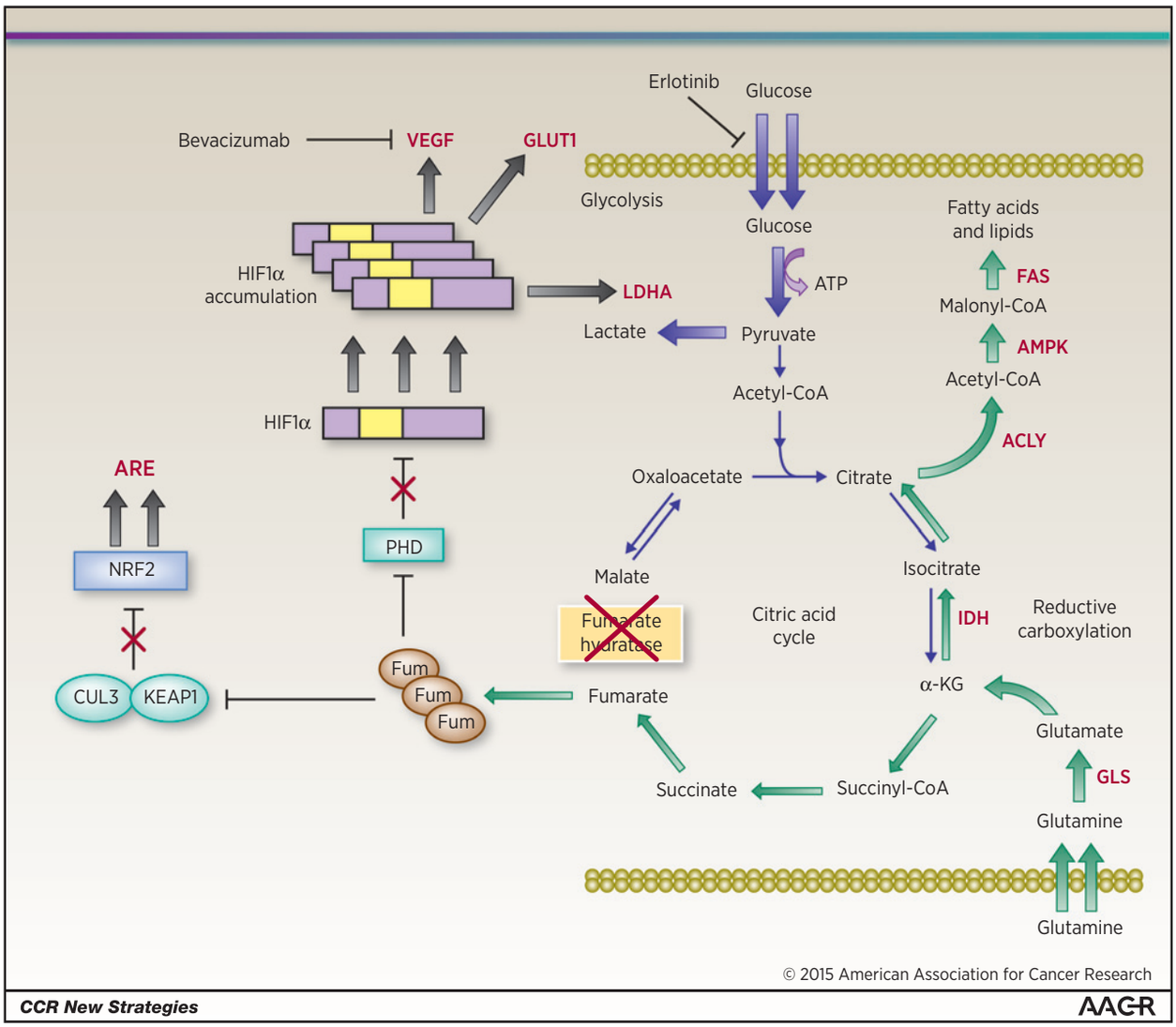

Figure 2.
Potential therapeutic targets for papillary type II RCC. Loss of fumarate hydratase (FH) is associated with the familial form of type II papillary RCC and results in the activation of several pathways. The loss of FH activity suppresses flow through the citric acid cycle in the normal direction and impairs the ability of the cell to use oxidative phosphorylation, forcing the cells to be dependent upon glucose and glycolysis for energy production. The loss of canonical tricarboxylic acid cycle function results in usage of the reductive carboxylation pathway and increased glutamine uptake for fatty acid synthesis and, in a similar manner to ccRCC, provides a series of potential targets for therapy (highlighted in red) involved in the conversion of glutamine to fatty acids. The highly increased levels of fumarate (Fum) result in inhibition of α-KG–dependent enzymes such as the PHDs. In normoxia, the PHDs hydroxylate the HIFα transcription factors to allow for VHL-dependent degradation. In FH-deficient RCC, the HIF1α accumulates and activates downstream targets. This results in increased levels of VEGF, lactate dehydrogenase A (LDHA) and the glucose transporter, GLUT1, that support the high levels glycolysis required by this aggressive form of RCC. A therapeutic approach utilizing the combination of bevacizumab and erlotinib is currently being evaluated. In addition, the elevated levels of fumarate result in succination of multiple proteins including the KEAP1 protein, which, as part of an E3 ubiquitin ligase complex with CUL3, targets the NRF2 transcription factor for degradation. Succination of KEAP1 inactivates KEAP1 and inhibits NRF2 degradation, resulting in activation of the NRF2 antioxidant response element (ARE) transcription pathway. While mutation of FH is rarely seen in sporadic type II papillary RCC, recent studies have reported activation of the NRF2 pathway in sporadic type II PRCC due to inactivating mutations of CUL3 or activating mutations of NRF2. An intense effort is under way to develop therapeutic agents that target the NRF2 ARE pathway.

acetyl CoA carboxylase, which would be expected to increase fatty acid synthesis. In addition, phospho-S6 ribosomal protein, a downstream mTOR effector, was also found to be activated in FH-deficient RCC cells (51, 67). Finally, FH-deficient RCC is characterized by a glutamine-dependent reductive carboxylation in which glutamine is the primary carbon source for lipid biosynthesis (Fig. 2; ref. 68).

KEAP1/Nrf2 antioxidant response pathway

As FH-deficient RCC is characterized by increased oxidative stress and elevated levels of reactive oxygen, effective antioxidant response is critical for continued growth (69). In FH-deficient RCC, increased levels of fumarate induce succination of the Kelch-like ECH-associated protein 1 (KEAP1), resulting in stabilization of nuclear factor E2-related factor 2 (Nrf2),

increasing expression of antioxidant response element (*ARE*) genes (Fig. 2; refs. 70, 71). Ooi and colleagues found a similar antioxidant response phenotype shared between hereditary and sporadic type II papillary RCC (71) and that CUL2 and Nrf2 mutations confer an Nrf2 activation phenotype in sporadic type II papillary RCC (72).

Therapeutic approaches for type II papillary RCC

A number of potential therapeutic approaches are currently being evaluated in preclinical as well as clinical trials. Screening studies are under way to identify agents that target Nrf2. The effect of agents such as metformin, which activate AMPK and its downstream target, SIRT1, on Nrf2 activity is also being evaluated. LDHA inhibition has been shown to have antitumor activity against FH-deficient RCC cells (73) and an effort is under way to identify clinically evaluable LDHA inhibitors. As mentioned above, FH-deficient RCC is characterized by increased levels of HIF1α, which results in increased expression of genes such as VEGF and the glucose transporter GLUT1, which are critical for the increased vasculature and glucose transport needed to support aerobic glycolysis (Fig. 2). To evaluate the effectiveness of agents such as bevacizumab and erlotinib, which could target the vasculature as well as impair glucose transport and glycolysis (74), a phase II trial of bevacizumab and erlotinib in patients with advanced HLRCC-associated type II papillary RCC as well as sporadic papillary RCC is currently under way (NCT01130519).

Summary

Although enormous progress has been made over the past 20 years in our understanding of the genetic basis and development of targeted therapeutics for ccRCC, we still have a long way to go. For ccRCC, we need better approaches for targeting both the VHL/HIF2α as well as chromatin-remodeling gene pathways. Given the profound genomic heterogeneity in ccRCC, it is most likely that successful strategies will need to target both truncal as well as branch driver mutations. Ongoing genomic studies in papillary RCC will hopefully identify novel driver mutations and pathways for this disease. For type I papillary RCC, the MET pathway seems particularly promising. For type II papillary RCC, targeting the tumor vascularity and glucose transport/glycolysis and targeting the KEAP1/NRF2 pathway also appear to be promising approaches. It is hoped that ongoing metabolomic and functional studies will identify novel metabolic pathways to target in both clear-cell and papillary RCC.

Authors' Contributions

Conception and design: R. Srinivasan, W.M. Linehan
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): W.M. Linehan

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