Targeting strategies in the treatment of fumarate hydratase deficient renal cell carcinoma

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Fumarate hydratase (FH) - deficient renal cell carcinoma (FHdRCC) is a rare aggressive subtype of RCC caused by a germline or sporadic loss-of-function mutation in the FH gene. Here, we summarize how FH deficiency results in the accumulation of fumarate, which in turn leads to activation of hypoxia-inducible factor (HIF) through inhibition of prolyl hydroxylases. HIF promotes tumorigenesis by orchestrating a metabolic switch to glycolysis even under normoxia, a phenomenon well-known as the Warburg effect. HIF activates the transcription of many genes, including vascular endothelial growth factor (VEGF). Crosstalk between HIF and epidermal growth factor receptor (EGFR) has also been described as a tumor-promoting mechanism. In this review we discuss therapeutic options for FHdRCC with a focus on anti-angiogenesis and EGFR-blockade. We also address potential targets that arise within the metabolic escape routes taken by FH-deficient cells for cell growth and survival.

KEYWORDS
hereditary leiomyomatosis and renal cell cancer, renal cell carcinoma (RCC), metabolism, glucose, fumarate hydratase deficient renal cell carcinoma, bevacizumab, erlotinib, fumarate hydratase

Renal cell carcinoma

Renal cell carcinoma (RCC) accounts for 2.6% of all cancers for almost 90% of malignant tumor formations of the kidney (1). The 2016 World Health Organization (WHO) Classification describes up to 14 known types of RCC, defining three main histological subtypes such as clear cell RCC (ccRCC), papillary RCC (pRCC) and chromophobe RCC (chRCC) (2). Additionally, a large spectrum of other renal tumors exists including a variety of
uncommon, sporadic and familial cancers, of which about 15% are benign such as oncocytoma, metanephric tumors or angiomyolipoma (2). In ccRCC, chromosome 3p deletions are found in about 70% to 90% (3). Furthermore, inactivation of the VHL gene has been demonstrated in 100% of familial ccRCC and in 57% of sporadic ccRCC (4). Two subtypes of pRCC are known, type I and II, with type I tumors having better survival than type II (5). Chromosomal gain of chromosomes 7 and 17 are the most consistent and characteristic found genetic alteration (6). Originating from distal convoluted tubules, chRCC is characterized by multiple chromosomal losses of chromosomes Y, 1, 2, 6, 10, 13q, 17, and 21 (7). Metabolic reprogramming appears to be a key core aspect in the development and progression of RCC. The VHL gene is an important regulator that is often inactivated and consequently involves HIF activation, which in turn stimulates the reprogramming of several metabolic pathways. Reprogramming of the glucose, fat and amino acid metabolism dysregulate the tricarboxylic acid cycle leading to a subsequent pro-oncogenic cellular environment. Effective inhibitors or drugs reversing reprogrammed metabolic pathways is the basis of new and currently more emerging targeted therapies against RCC (8).

RCC metabolism

Metabolism of glucose, amino acids and lipids is a key determinant of tumor growth (1 9). RCC is a metabolically very active tumor. The key factors which are closely linked with tumor microenvironment (TME) metabolic alterations in RCC include HIF, fatty acid synthase (FASN) and pyruvate kinase 2 (PKM2), being potential targets in cancer therapy (10). Glycolysis and hypoxia play a key role in the failure of RCC therapy. ccRCC shows disturbed metabolism of glucose, amino acids and lipids (11, 12), driving tumor growth and affecting prognosis (13). Moreover, the significant feature of most ccRCCs is the loss of VHL, which causes HIF accumulation and drives the cellular hypoxic response. Consecutively, significant variations in metabolism have been found in ccRCCs (13, 14). In addition, molecular patterns involving metabolic pathways correlated with worse survival in ccRCC, including downregulation of AMP-activated kinase (AMPK) complex and the Krebs cycle genes, upregulation of genes involved in the pentose phosphate pathway and fatty acid synthesis (15). Functional deficiency of succinate dehydrogenase (SDH), resulting in succinate accumulation, is a common feature in up to 80% of ccRCCs (16). Survival analyses of the TCGA-KIRC dataset confirmed poor survival rates in case of lower expression of the SDH subunits SDHB, SDHC and SDHD (16). Notably, RCC tumors show a remarkable metabolic heterogeneity (17, 18), however, still little is known how specific cellular components of the TME contribute to therapy response or resistance mechanisms.

Modulation of metabolic ‘checkpoints’ driven by HIF and other factors may provide a new therapeutic strategy to reverse TME features responsible for RCC drug resistance. Metabolic changes including enhanced glycolysis and glutaminolysis as well as increased antioxidant activity have been associated with TKI resistance in RCC (19). The frequency of the metabolic reprogramming may render RCC a suitable disease for the investigation of potential novel therapeutic agents that target tumor metabolism.

Fumarate hydratase deficient renal cell carcinoma (FHdRCC)

FHdRCC is a rare and aggressive subtype of type 2 papillary RCC, mainly affecting younger patients, metastasizing early from small solitary lesions. It is caused by an inactivating mutation of the FH gene with a 15% lifetime risk for FH mutation carriers to develop RCC (20). Conversely, restoration of FH activity has been shown to stop development of FHdRCC and to restore the physiological metabolic phenotype in an animal model (21). FHdRCC can be associated with the hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome, an autosomal-dominant hereditary syndrome with predisposition to develop smooth muscle tumors of the skin and uterus. In the largest case series of HLRCC including 185 patients from 69 families, 12.4% developed RCC resulting in a lifetime risk of 21%. Although RCC occurs only in a minority of cases, it presents highly aggressive with poor survival in symptomatic patients with stage 3 to 4 disease. Thus, renal imaging screening should be offered to these patients resulting in earlier-stage diagnosis of RCC with consecutive survival benefit (20). The WHO lists the subtype ‘HLRCC-associated RCC’ in their RCC classification, which strictly speaking defines a group of genetically altered types of FHdRCC. The more general term FHdRCC should rather be used to include both hereditary and known sporadic forms of cancer development (22). FHdRCC shows histopathological features such as papillary architecture with tubule cystic growth patterns, abundant eosinophilic cytoplasm, perinucleolar halos and as shown recently, tumor cannibalism and lymphocytic emperipolesis (23). Yet, it still remains difficult to distinguish FHdRCC from papillary RCC by means of solely pathological criteria.

Metabolic changes, epigenetic and signalling pathway alterations and therapeutic targets in FHdRCC

FH (EC 4.2.1.2) participates in the mitochondrial TCA cycle, which serves the production of cellular energy production in the form of ATP through oxidative phosphorylation (OXPHOS). FH catalyzes the conversion of fumarate to L-malate. Fumarate dehydrogenase (16). The TCA cycle is fueled by acetyl-CoA,
which condenses with oxaloacetate to form citrate in the first step of the pathway. To keep the TCA cycle going, acetyl-CoA must therefore continuously be generated either by oxidative decarboxylation of pyruvate, by oxidation of long-chain fatty acids, or by oxidative degradation of certain amino acids. When glucose levels are low or TCA cycle intermediates are diverted for biosynthetic purposes, cells use glutaminolysis to maintain the TCA cycle. In this pathway, glutaminase breaks down glutamine to form glutamate, which is further converted to α-ketoglutarate (α-KG). Of note, glutamine replenishment of the TCA cycle can result in fumarate accumulation even in FH-containing cells (24). An exciting study by Frezza et al. has addressed the mechanism that allows FH-deficient immortalized kidney cells to survive without a functional TCA cycle. By combining gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and a computer model of metabolism, the authors found that FH-deficient cells used glutamine to survive and that accumulating fumarate was mainly glutamine-derived (24-26).

In a cataplerotic pathway, FH-deficient cells use the accumulated TCA cycle intermediate succinate to initiate porphyrin biosynthesis via succinyl-CoA. However, the resulting heme, i.e. iron protoporphyrin IX, is immediately degraded again by heme oxygenase (HMOX) and other enzymes. This apparently futile cycle of concomitant haem biosynthesis and degradation, enables FH-deficient cells to generate at least some mitochondrial NADH. The HMOX inhibitor zinc protoporphyrin or HMOX silencing impaired FH-deficient cell growth and colony formation. Likewise, inhibition of haem biosynthesis using hemin, an approved drug used for acute porphyria, reduced colony formation of FH-deficient cells, altogether indicating that cells lacking FH critically depend on this unusual pathway and that haem biosynthesis and degradation pathways may be attractive targets in the treatment of FHdRCC (24, 26). Yet another pathway that increases cellular levels of fumarate is the urea cycle, which converts toxic ammonia to urea for subsequent excretion. Ammonia mainly accumulates during amino acid catabolism. Fumarate is produced in the 4th step of the cycle, when argininosuccinate is converted to arginine. Intriguingly, FH-deficient cells reverse this step and use fumarate and arginine to form argininosuccinate. Reversal of this metabolic step appears to be critical, since arginine depletion impaired cell proliferation and survival of FH-deficient cells (27). Therefore, arginine deprivation or targeting argininosuccinate synthase should be beneficial in this particular subtype of RCC (28).

It is not surprising that the lack of FH changes many cellular functions in FHdRCC. Fumarate and its immediate precursor succinate can act as oncometabolites by competitively inhibiting α-ketoglutarate (α-KG)-dependent dioxygenases, a family of enzymes that also includes prolyl hydroxylases. Suppression of protein prolyl hydroxylation slows down the degradation of HIF (21) and HIF stabilization in turn promotes tumorigenesis through transcriptional activation of pro-angiogenic genes including vascular endothelial growth factor (VEGF). HIF also activates glycolytic genes that contribute to the Warburg effect, a metabolic shift to aerobic glycolysis in normoxia. In addition to prolyl hydroxylase, fumarate and succinate inhibit other α-KG-dependent dioxygenases. Accumulating fumarate and succinate for instance suppress histone and DNA demethylases by depletion of α-KG causing genome-wide epigenetic modifications that further contribute to tumorigenesis (29). The mechanism of α-KG inhibition through accumulating fumarate is still debated, literature to date refers to this as a competitive inhibition process (30). For example, the histone de-methylation process is inhibited resulting in hypermethylation at histones H3K9 and H3K27 (31). Thus, transcription of tumor suppressor genes and differentiation genes is inhibited contributing to cell dedifferentiation, tumor progression and drug resistance (31). A TCGA pan-Kidney Cancer Analysis of 843 RCC confirmed distinctive features of each RCC subtype. FHdRCC was characterized by a CpG island methylator phenotype, DNA hypermethylation/CDKN2A alterations and increased immune signature expression for select immune gene signatures, including Th2 gene signature (similar to ccRCC) (32). In detail, poor outcome was linked in all RCC subtypes with hypermethylation of WNT pathway regulatory genes (SFRP1 and DKK1), suggesting that hypermethylation of SFRP1 and DKK1 might be a promising prognostic biomarker in RCC (32). These observations may provide the therapeutic rationale of introducing immune checkpoint inhibitors, CDK4/6 inhibitors (33) and de-methylating agents in patients with FHdRCC.

In cancer cells with FH deficiency, fumarate can also react with cysteine residues of proteins in a non-enzymatic manner, generating S-(2-succinyl)cysteine. Such a protein modification, which is known as cysteine succination, eliminates the ability of Kelch-like ECH-associated protein 1 (KEAP1) to repress nuclear factor (erythroid-derived 2)-like 2 (NRF2) (34). Although activation of NRF2, a master regulator of antioxidant responses, in cancer can be beneficial, the fumarate-induced stabilization of NRF2 in HLRCC facilitates tumor growth and survival (34). SMARCC1 is a core member of the tumor suppressing SWI-SNF chromatin remodelling complex and also affected by succination. In ccRCC, SMARCC1 is commonly deleted because of its position on chromosome 3, which is known as a potentially tumor-promoting region in RCC. Using a fumarate-competitive chemoproteomic probe in concert with LC-MS, a recent study has identified a novel FH-regulated cysteine residue in SMARCC1 which is subject to succination (35). As a consequence, SWI-SNF complex formation is impaired and thus its tumor-suppressing activity is reduced.

In certain tumors, HIF stabilization and accumulation can also be induced by activation of epidermal growth factor receptor (EGFR) (36), EGFR signalling can increase the levels of HIF under normoxic conditions through the
phosphoinositide 3-kinase (PI3K)/AKT pathway. EGFR can thus promote the Warburg effect. Consistent with EGFR-mediated HIF stabilization, EGFR inhibitors such as erlotinib can decrease the expression of the HIF target VEGF (37). Accordingly, resistance to EGFR inhibitors can be associated with increased levels of VEGF in the tumor microenvironment. Altogether, these observations have led to the concept of combining EGFR and VEGF(R) inhibitors (38). However, there are currently no reports about alterations of EGF signalling in FH-deficient tumors supporting the combined application of EGFR and VEGF(R) inhibitors.

The metabolic shift towards glycolysis in FHdRCC has been shown to lower the levels of AMPK (21). AMPK is a highly conserved metabolic sensor that governs cellular adaptation to energy deficiency and environmental stress. AMPK acts as a metabolic tumor suppressor by activating p53 and by regulating mammalian target of rapamycin (mTOR). The attenuation of AMPK in FHdRCC facilitates the activation of mTOR, which then promotes the biosynthetic pathways required for cell proliferation. Epidemiological studies indicate that the incidence of cancer is reduced in type 2 diabetes treated with the AMPK activator metformin (39). Therefore, pharmacological re-activation of AMPK using metformin would in principle also be a promising approach in the treatment of FHdRCC. However, metformin-induced AMPK activation occurs via inhibition of respiratory chain Complex I. By inhibiting mitochondrial OXPHOS, ATP is depleted, ultimately resulting in AMPK activation (40). Considering that FH deficiency itself causes a strong suppression of the mitochondrial respiration, it is currently unclear whether treatment with metformin would indeed be beneficial (26, 41).

Clinical trials in HLRCC and FHdRCC

In metastatic FHdRCC, therapy regimens with immune checkpoint inhibitors (ICI), mTOR inhibitors, multi-target tyrosine kinase inhibitors (TKI) and various combinations have been tested in the past. PD-1/PD-L1 expression in tumor cells and tumor-infiltrating lymphocytes have been found only in a small proportion of FHdRCC cases. Since ICIs failed to induce satisfactory response rates, they should only be offered to patients with a PD-L1 positive tumor (42, 43). Dual inhibition of mTOR and VEGF was reported with ORR rates of up to 44%, whereas patients treated with mTOR inhibition alone showed no response (43, 44). TKIs were superior to mTOR inhibitors or ICIs alone, presenting with an ORR of up to 64% and a time to progression of 11.6 months (44). Cabozantinib, which is approved for metastatic RCC, might be the preferred TKI in HLRCC (45). In the AVATAR trial (NCT01130519), the combination of bevacizumab and erlotinib has shown promising preliminary results in HLRCC patients (46). In comparison to sporadic papillary RCC, the HLRCC cohort benefited with an ORR of 72% and a median PFS of 21.1 months versus 35% and 8.8 months, respectively (46). Findings from a first-line setting of this combination revealed that FHdRCC patients treated with bevacizumab and erlotinib showed an ORR of 50% with a median PFS of 13.3 months and an impressive disease control rate of 90% (47). Pharmacological re-activation of AMPK using metformin may also be a promising approach in the treatment of FHdRCC. The combination of the VEGF/EGFR inhibitor vandetanib with metformin has already been tested in a phase II study (NCT02495103) but was not continued due to the lack of vandetanib availability. All registered studies in patients with FHdRCC are summarized in Table 1.

### Outlook

The future agenda may involve targeting of cancer cell metabolism at the level of glutaminolysis (48), to slow down TCA cycle activity and to avoid fumarate accumulation (Figure 1). The strict arginine dependence of FH-deficient cells also suggests arginine deprivation as an appropriate therapeutic approach (27). Moreover, OXPHOS, which

### Table 1 Ongoing clinical trials in HLRCC and FHdRCC

| Study number | Agents | Phase | Primary endpoint | Therapy setting | Status |
|--------------|--------|-------|-----------------|----------------|--------|
| NCT04068831 | talazoparib + avelumab | II | ORR | second line | recruiting |
| NCT04387500 | sintilimab + axitinib | II | PFS, ORR | first line | recruiting |
| NCT04146831 | sintilimab | II | PFS | second line | not yet recruiting |

CR, complete response; ORR, objective response rate; PFS, progression-free survival; MTD, maximum tolerated dose. Status according to clinicaltrials.gov assessed on 08th February 2022.
generates ATP in a TCA cycle dependent manner, can be targeted using drugs such as metformin or possibly also atovaquone, and arsenic trioxide (50) to revert the tumorigenic metabolism through AMPK activation (50), provided that side effects are manageable. Gene therapy-based replacement of defective enzymes in target tissues, which is a current effort in the treatment of metabolic diseases (51), may be an ultimate goal help to improve the therapeutic opportunities of FH-deficient
RCC. Moreover, targeting the epigenetic machinery to decrease the oncogenic impact of metabolic changes may be effective also in FHdRCC patients (31).

Take home messages

In FHdRCC, the oncometabolite fumarate activates HIF-1α and promotes the tumorigenic Warburg effect. Anti-VEGF antibody bevacizumab plus EGFR inhibitor erlotinib showed response rates up to 72%. Targeting tumor metabolism aimed at AMPK re-activation may further improve FHdRCC therapy. Given the metabolic escape routes in FH-deficient cells, inhibition of haem biosynthesis and/or degradation as well as arginine deprivation offer novel therapeutic opportunities in FHdRCC. Additionally, DNA CpG islands (CGI) hypermethylation status might be a cornerstone of introducing de-methylating agents in the therapeutic landscape of FHdRCC.

Conclusions

In conclusion, FHdRCC is a genetically or sporadically acquired aggressive variant of type 2 papillary kidney cancer that affects younger patients. Understanding the metabolic changes and signalling pathway alterations caused by FH deficiency may facilitate the development of more effective therapies for this aggressive and mostly fatal disease. Since the incidence of FHdRCC is low, it does not seem justified to screen all RCC patients for genetic abnormalities in the FH gene. However, it is important to raise awareness for clinical correlations and morphological signs to initiate further molecular and germline analysis. Long-lasting partial responses and manageable low-grade toxicities suggest that the combination therapy with bevacizumab and erlotinib should be considered as standard first-line therapy in locally advanced/metastatic FHdRCC.

Author contributions

Conceptualization, AL, RP, and MT. Methodology, AL and GT. Data and literature curation, AL, GT, RP, MT, and AS. Writing—original draft preparation, AL, RP, and MT. Writing—review and editing, AS, GT, PS, and MT. Supervision, MT, PS, and AS. Project administration, GT and AS. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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