ONCONEPHROLOGY

Oncometabolites in renal cancer

Cissy Yong[1,2], Grant D. Stewart[1,2] and Christian Frezza[3]**

Abstract | The study of cancer metabolism has evolved vastly beyond the remit of tumour proliferation and survival with the identification of the role of 'oncometabolites' in tumorigenesis. Simply defined, oncometabolites are conventional metabolites that, when aberrantly accumulated, have pro-oncogenic functions. Their discovery has led researchers to revisit the Warburg hypothesis, first postulated in the 1950s, of aberrant metabolism as an aetiological determinant of cancer. As such, the identification of oncometabolites and their utilization in diagnostics and prognostics, as novel therapeutic targets and as biomarkers of disease, are areas of considerable interest in oncology. To date, fumarate, succinate, L-2-hydroxyglutarate (L-2-HG) and D-2-hydroxyglutarate (D-2-HG) have been characterized as bona fide oncometabolites. Extensive metabolic reprogramming occurs during tumour initiation and progression in renal cell carcinoma (RCC) and three oncometabolites — fumarate, succinate and L-2-HG — have been implicated in this disease process. All of these oncometabolites inhibit a superfamily of enzymes known as α-ketoglutarate-dependent dioxygenases, leading to epigenetic dysregulation and induction of pseudohypoxic phenotypes, and also have specific pro-oncogenic capabilities. Oncometabolites could potentially be exploited for the development of novel targeted therapies and as biomarkers of disease.

Cancers of the kidney account for an estimated 2.2% of the global burden of all cancers, which translates into more than 400,000 new diagnoses worldwide in 2018 (REF.[1]). Renal cell carcinoma (RCC), a cancer of the kidney parenchyma, is the most common solid tumour of the kidney and the most lethal of all urological malignancies[2]. Almost a third of all patients with RCC have metastatic dissemination at presentation and nearly half of all patients die from their disease[1,2]. RCC is increasingly recognized as a collection of renal cancer subtypes, each with distinct histology, genetic and molecular alterations, clinical course and therapeutic responses[3–5].

Single-cell sequencing studies have shed light on the oncogenic events and cells of origin of two common RCC subtypes, clear cell RCC (ccRCC) and type 1 papillary RCC (pRCC), suggesting that these tumours might arise from convoluted proximal tubular cells with divergent fates[6]. The TRACERx Renal studies, published in 2018, expanded our knowledge of the role of genomics in RCC tumour evolution[7–9]. Loss of chromosome 3p, a pathognomonic feature of ccRCC occurring in >90% of patients[10,11], was typically found to be the initiating driver event in sporadic ccRCC[7]. This event occurred as early as childhood in only a few hundred cells, preceding cancer diagnosis by up to 3–5 decades. The *VHL* gene, which encodes the von Hippel–Lindau disease tumour suppressor, and the chromatin-modifying genes *PBRM1, BAP1* and *SETD2*, are co-located in chromosome 3p. Perhaps unsurprisingly, mutations in these genes are the most prevalent somatic gene perturbations found in ccRCC, as patients with loss of chromosome 3p are rendered vulnerable to complete (biallelic) inactivation of these genes during their lifetime[7,10,12]. The TRACERx Renal studies also identified distinct evolutionary subtypes of ccRCC that correlate with clinical phenotypes and outcomes and, therefore, could be used to guide intervention and surveillance[8,9]. As genomic technology advances, the genetic perturbations implicated in ccRCC continue to expand and include somatic mutations in *TERT*[7,13], *PTEN*[10,12], *MYC*[14,15] and *mTOR*[12] signalling pathways, as well as in numerous metabolic pathways[10]. Subtype-specific genetic perturbations have also been identified, such as mutations in fumarate hydratase (FH) in type 2 pRCC[16].

RCC is increasingly recognized as a disease of cell metabolism. Before the advent of the omics era, at least 12 genes implicated in RCC pathogenesis were identified as having roles in fundamental metabolic processes[17,18]. One classic example in RCC is the ability of *VHL* inactivation to rewire the normal metabolic adaptation response to oxygen deprivation. In ccRCC, inactivation of *VHL* leads to the aberrant accumulation of the transcription factors hypoxia-inducible factor 1α (HIF1α) and HIF2α despite normoxia[19,20], resulting in upregulation of pathways involved in glycolysis, fatty acid synthesis and glycogen synthesis[21–23]. Metabolic reprogramming that facilitates the capacity of the neoplasm to meet its

[1]*Department of Surgery, University of Cambridge, Cambridge Biomedical Campus, Cambridge, UK.*

[2]*Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK.*

[3]*Medical Research Council Cancer Unit, University of Cambridge, Cambridge Biomedical Campus, Cambridge, UK.*

**e-mail: gds35@cam.ac.uk; cf366@mrc-cu.cam.ac.uk*

https://doi.org/10.1038/s41581-019-0210-z

Key points

- Oncometabolites are aberrantly accumulated metabolites that possess pro-oncogenic capabilities, they contribute to tumorigenesis via epigenetic dysregulation and can influence tumour progression through phenotypic switches such as epithelial to mesenchymal transition.
- L-2-hydroxyglutarate, fumarate and succinate are bona fide renal cell carcinoma (RCC) oncometabolites; exploitation of these oncometabolites and their downstream signalling effects are attractive targets for novel therapies and as biomarkers of disease.
- Oncometabolites have shared pro-oncogenic functions owing to their ability to inhibit α-ketoglutarate-dependent dioxygenases as well as individual oncometabolite-specific functions.
- Chromatin remodelling via oncometabolites may recapitulate the effects of other epigenetic modifiers mutated in RCC, thus converging on the same gene signature; identification of the underlying pathways influences treatment strategy.
- Elucidation of exogenous factors that give rise to oncometabolite production, such as hyperglycaemia, may prove to be a synergistic strategy to reduce the levels of oncometabolites and their subsequent sequelae.

bioenergetic demands, including uncontrolled proliferation and the acquisition of other hallmark traits of cancer, is now recognized as having a fundamental role in the malignant transformation of cells and in the phenotypic evolution of tumours[24,25]. HIF has also been identified as a common target for metabolic reprogramming in RCC owing to genetic perturbations that affect the genes encoding FH[26–28], succinate dehydrogenase (SDH)[26,27,29], the tuberous sclerosis complex[30] and fructose-1,6-bisphosphatase 1 (FBP1)[31]. FBP1 is a key gluconeogenic enzyme that has been shown to directly interact with HIFs to restrain its transcriptional activity[31]. The discovery that FPB1 is ubiquitously suppressed in ccRCC[31] further supports the classification of RCC as a metabolic disease and the identification of HIF as a common denominator that is associated with multiple RCC subtypes underlies its potential as a key candidate for RCC therapies.

Further corroboration of the key role of metabolite reprogramming in RCC was provided by the landmark Cancer Genome Atlas (TCGA) integrated platform analyses that studied the genome, transcriptome and proteome of more than 400 ccRCC tumours[10]. This study highlighted the extensive metabolic reprogramming that occurs in ccRCC, including upregulation of fatty acid synthesis, the pentose phosphate pathway and glutamine transporters, and downregulation of the tricarboxylic acid (TCA) cycle (FIG. 1). This reprogramming correlated with more aggressive disease and worse prognosis[10]. Downregulation of lipid degradation (β-oxidation pathway) also correlated with poor prognosis in ccRCC[32]. Subtype-specific metabolic gene alterations that correlated with disease aggression and patient survival were identified in subsequent TCGA studies across the three major RCC subtypes (ccRCC, pRCC and chromophobe RCC), supporting the principle of subtype-specific management and providing potential subtype-specific targets for novel therapies[5].

Given the metabolic nature of RCC and the emergence of metabolic reprogramming as a contemporary hallmark of cancer[33], the field of cancer metabolomics has rapidly developed over the last decade. In general, metabolomics encompasses the ability to globally detect the metabolites present in a system (cell, tissue or organism) under a given set of conditions[34]. Crucially, metabolomics can also capture the underlying environmental influences and external perturbations of a system[34,35]. As the tumour microenvironment has a profound effect on metabolism[36,37], integrating metabolomics with other 'omics' studies enables a holistic approach to understanding cancer pathophysiology.

A handful of metabolomic studies in RCC have been performed to date. The largest of these studies profiled a single cohort of 138 patients with ccRCC[38,39] and broadly corroborated several findings from the TCGA study[10]. They identified a network of metabolic shifts involving upregulation of glycolysis, the pentose phosphate pathway and glutamine uptake that correlated with disease aggressiveness[38,39]. However, integration of these metabolomic data with the TCGA dataset highlighted a lack of linear correlation between enzyme expression and the levels of corresponding catalysed metabolites[38]. This lack of linearity has been theorized to result from the shunting of metabolites into alternative cancer-reprogrammed pathways[38,40]. Overall, these studies suggest that physiological metabolism might be efficiently manipulated by RCC to provide the conditions needed for cancer cells to survive and proliferate.

The identification of key genetic mutations in genes that encode enzymes with roles in mitochondrial metabolism such as FH and SDH in cancer cells[16,41,42] paved the way for the discovery and evolution of the oncometabolite paradigm. Oncometabolites are defined as conventional metabolites that when aberrantly accumulated have pro-oncogenic capabilities that can contribute to tumorigenesis via epigenetic dysregulation, as well as influence tumour phenotype and progression. Three of the established bona fide oncometabolites — fumarate, succinate and L-2-hydroxyglutarate (L-2-HG) — have been implicated in hereditary and sporadic subtypes of RCC. This observation, coupled with the strong metabolic paradigm in RCC and the extensive metabolic reprogramming that underpins RCC tumour progression, commands attention to this growing area of crossover research in oncology and metabolism. Here, we review the role of oncometabolites in RCC, their origins and downstream effects and their potential applications as novel therapeutic targets and biomarkers of disease.

Origin of the oncometabolite paradigm

The inception of the oncometabolite paradigm predates the use of this term in the literature. One could argue that the concept began with Otto Warburg's hypothesis of aberrant metabolism as an aetiological determinant of cancer[43,44]. Based on the observation of excessive fermentation of glucose in mammalian cancer cells irrespective of the presence of oxygen, later termed the Warburg effect[45] (FIG. 1), Warburg postulated that this abnormal compensatory mechanism counteracted an irreversible injury of cellular respiration and induced an undifferentiated state, giving rise to "cells that grow wildly — the cancer cells"[43,44]. He suggested that the prime cause of cancer might be mitochondrial dysfunction. Warburg's hypothesis was soon dismissed, however, owing to the discovery of mutated oncogenes and tumour suppressor

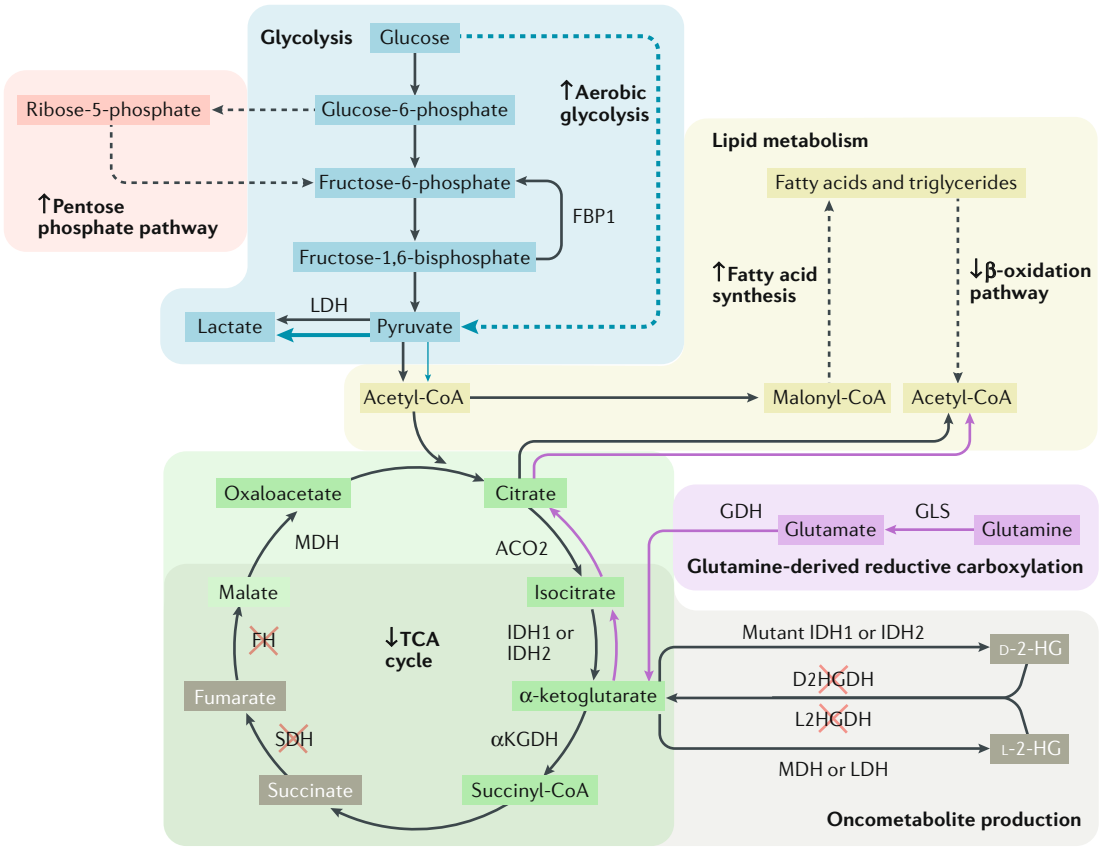

Fig. 1 | **Key metabolic pathways in renal cell carcinoma.** Glycolysis catabolises glucose to form pyruvate and yields intermediates for entry into the pentose phosphate pathway, the tricarboxylic acid (TCA) cycle and lipid synthesis. The Warburg effect — upregulation of glycolysis even in the presence of oxygen — is observed in many cancers and correlates with poor outcomes in patients with clear cell renal cell carcinoma (ccRCC)[10]. The pentose phosphate pathway provides reducing equivalents (NADPH) and precursors for nucleotide synthesis. Upregulation of this pathway is associated with aggressive ccRCC and poor patient outcomes[10]. The TCA cycle is a series of reactions that fully oxidize carbohydrates, lipids and proteins and generate reducing equivalents (NADH) for the electron transport chain to generate ATP. The TCA cycle intermediates are also a source of precursors for lipid and amino acid biosynthesis. Downregulation of TCA cycle genes correlates with aggressive ccRCC and poor patient outcomes[10]. Lipid synthesis is required for energy stores and synthesis of cell membrane components, whereas lipid degradation via the β-oxidation pathway is required for release of energy stores and to generate acetyl-CoA, which then feeds the TCA cycle. In RCC, downregulation of β-oxidation is associated with poor patient outcomes[32]. Upregulation of fatty acid synthesis also correlates with aggressive disease and poor outcomes in RCC[10]. Reversed flow of the canonical TCA cycle (known as reductive carboxylation, purple arrows) enables glutamine-derived α-ketoglutarate to be metabolized to form citrate, which can generate lipogenic acetyl-CoA once exported into the cytosol. Cancer cells with mitochondrial defects, such as fumarate hydratase (FH)-deficient and succinate dehydrogenase (SDH)-deficient RCC, predominantly utilize this pathway to support cell growth[175,176]. Upregulation of glutamine transporters correlates with aggressive disease and poor outcomes in patients with RCC[10]. Loss-of-function mutations in genes that encode the TCA cycle enzymes FH and SDH lead to accumulation of the oncometabolites fumarate and succinate, respectively. 2-hydroxyglutarate (2HG) exists in two isoforms (D-2-HG and L-2-HG), both of which are oncometabolites. Gain-of-function neomorphic activity of isocitrate dehydrogenase 1 (IDH1) or IDH2 leads to accumulation of D-2-HG, whereas promiscuous activity of malate dehydrogenase (MDH) and/or lactate dehydrogenase (LDH) results in the accumulation of L-2-HG. Loss-of-function of the enzymes D-2-hydroxyglutarate dehydrogenase (D2HGDH) and L-2-hydroxyglutarate dehydrogenase (L2HGDH), which catalyse the oxidation of D-2-HG and L-2-HG to α-ketoglutarate, also result in the accumulation of D-2-HG and L-2-HG, respectively. αKGDH, α-ketoglutarate dehydrogenase; ACO2, aconitase; CoA, coenzyme A; FBP1, fructose-1,6-bisphosphatase 1; GDH, glutamate dehydrogenase; GLS, glutaminase.

Hereditary leiomyomatosis and renal cell cancer (HLRCC). An autosomal dominant hereditary cancer syndrome caused by germline mutations in *FH*. HLRCC is characterized by cutaneous and uterine leiomyomas and is associated with papillary type 2 RCC.

Hereditary paraganglioma (PGL). A dominantly inherited rare condition consisting of benign tumours arising from neuroendocrine tissues, typically in the head and neck.

genes, and altered metabolism was postulated to be a bystander effect secondary to the genetic perturbations that were identified in numerous cancers[46–49].

In the early 2000s, Warburg's hypothesis came full circle when several genetic perturbations in genes encoding two key enzymes in the TCA cycle, *FH*[16] and *SDH*[41,42,50], were implicated in the development of hereditary leiomyomatosis and renal cell cancer (HLRCC) and hereditary paraganglioma (PGL), respectively. Subsequent seminal studies uncovered unconventional and novel roles of fumarate and succinate in deregulating the HIF pathway through direct inhibition of prolyl hydroxylases (PHDs), which have roles in targeting HIF for degradation[26,27,29]. Stabilization of HIF1α and HIF2α alongside upregulation of downstream HIF1 products, such as vascular endothelial growth factor (VEGF)

and glucose transporter type 1 (GLUT1), were observed in HLRCC tumours, SDH-deficient PGLs and associated phaeochromocytomas (PCCs) in the absence of *VHL* inactivation[26,27]. These findings support a role of aberrant accumulation of fumarate and succinate in creating the hypoxic signatures and highly vascularized phenotype that are characteristic of these tumours[51–55]. This pseudohypoxic phenotype is also characteristic of *VHL*-mutant RCC and is associated with tumour aggression[56,57].

Dang et al. were the first group to coin the term 'oncometabolite' to describe the potential tumorigenic role of pathological accumulation of the metabolite D-2-hydroxyglutarate (D-2-HG)[58]. In physiological settings, 2-hydroxyglutarate (2HG) exists in two natural isoforms: L-2-HG and D-2-HG. These isoforms are minor metabolic by-products that are produced via distinct biological mechanisms and are normally kept at unappreciable levels by conversion back to α-ketoglutarate (αKG, also known as 2-oxoglutarate) via their respective hydroxyglutarate dehydrogenase enzymes (L2HGDH and D2HGDH)[59–62]. Dang et al. reported abnormally elevated levels of D-2-HG (up to tens of micromoles per gram of tissue) in patients with malignant gliomas harbouring a single mutant copy of *IDH1*, which encodes isocitrate dehydrogenase (IDH)[58]. These levels were >100-fold greater than those in malignant gliomas with wild type *IDH*. IDH is the TCA cycle enzyme that is responsible for the reversible oxidative carboxylation of isocitrate to αKG. Mutation of *IDH1* confers a gain-of-function neomorphic activity of IDH that catalyses the reduction of αKG to D-2-HG, leading to its accumulation[58]. In patients with inborn errors of 2HG metabolism, elevated levels of L-2-HG have been associated with brain tumours[63,64], as well as with a case of Wilms tumour (nephroblastoma)[63,65]; D-2-HG accumulations have not been associated with cancer in these patients[66].

A myriad of oncometabolite-focused studies have expanded upon these initial findings, establishing a core group of bona fide oncometabolites: fumarate, succinate, L-2-HG and D-2-HG[47,60,67,68]. These oncometabolites are increasingly associated with numerous malignancies, including neuroendocrine tumours[50,69,70], brain tumours[71,72], haematological malignancies[73,74], head and neck squamous cell carcinoma[75] and hereditary and sporadic forms of RCC[60,76–79].

Endogenous origins of oncometabolites

Identification of loss-of-function mutations in genes encoding the key TCA cycle enzymes SDH and FH, which lead to the accumulation of succinate and fumarate, respectively, as well as a gain-of-function mutation in IDH, which results in the accumulation of D-2-HG, led to an appreciation of how these genetic perturbations act as tumour suppressor genes and oncogenes[27,47,67,80]. Mutations in both *FH* and *SDH* subunits in tumours follow Knudson's 'two hit' hypothesis of tumorigenesis[81]. In patients with heterozygous germline mutations in *SDH* or *FH* (that is, inheritance of one mutated allele), loss of heterozygosity (LOH) (that is, loss of the remaining wild type allele) seems to be the factor that leads to tumorigenesis[47,67,80].

SDH is composed of four subunits (SDHA, SDHB, SDHC and SDHD) and two assembly factors (SDHAF1 and SDHAF2), each of which is encoded by distinct genes across multiple chromosomes[82]. LOH in multiple subunits of *SDH* predisposes to a variety of cancers, including PGL and SDH-deficient RCC, which is a very rare (0.2% of all RCCs) and aggressive disease with early onset (mean age 37–46 years)[5,83–87]. The majority of tumours harbour *SDHB* mutations (82%), although mutations in all four SDH subunits and in the assembly factor SDHAF2 have been implicated in the pathogenesis of RCC[83,86–90]. Moreover, increased expression of *SDHB*, *SDHC* and *SDHD* correlate with better survival outcomes in patients with ccRCC[10]. LOH in patients with heterozygous *FH* germline mutations predisposes to HLRCC[16,91,92], which is associated with type 2 pRCC in up to 18% of patients[93,94]. HLRCC-associated renal tumours are also highly aggressive, with an early age of onset (mean 10–44 years)[94,95]. Interestingly, mutations in *SDH* or *FH* both predispose to the development of PGL and PCC[69,83,84,96,97].

Distinct clinical phenotypes are also observed in FH-deficient and SDH-deficient diseases. Homozygous germline mutations in *FH* give rise to fumaric aciduria, a rare metabolic disease associated with infantile encephalopathy, brain malformations and neonatal polycythaemia without an associated cancer predisposition[98,99], whereas homozygous germline mutations of *SDHA* cause severe neurological dysfunction and cardiomyopathy[84]. This divergence in clinical phenotypes suggests that the 'two-hit' mutational timing and tissue-specific nature of mutations may be crucial to cancer predisposition. As such, it has been suggested that oncometabolites might be insufficient for oncogenic transformation[60]. Potentially confounding this notion is the finding that patients with inborn errors of metabolism, such as fumarate aciduria, often do not survive long enough for potential malignancies to manifest[100].

The mechanisms that drive the development of cancer in specific tissues upon LOH are not yet understood. Building on current hypotheses[101], we postulate a concept of LOH tolerance in permissive tissues that enable tumorigenesis owing to their capability to metabolically adapt and/or compensate for genetic perturbations. For example, reversal of the activity of the urea cycle enzyme arginosuccinate lyase (ASL) in FH-deficient cells enables their survival by funnelling accumulated fumarate into aberrant urea cycle metabolism[102]. By contrast, intolerance of LOH in a small proportion of cells and/or tissues might result in lethality, thereby terminating the propagation or replication of genetic perturbations and thus attenuating tumorigenesis. Further understanding of the mechanisms that lead to distinctive patterns of cancer development may identify tissue-specific vulnerabilities that could have a great impact on the future management of these clinically challenging diseases.

In contrast to *SDH* and *FH*, *IDH1* and *IDH2*, which encode the compartment-specific isoforms of IDH in the cytosol and mitochondria, respectively[103], express a dominant pattern of oncogenic behaviour. Somatic mutations in only one copy of the *IDH* gene (one wild type copy is retained) that confer the neomorphic gain-of-function activity that converts αKG into D-2-HG[58] have been

Phaeochromocytomas (PCCs). A type of paraganglioma that arises from the adrenal glands and produces catecholamines such as adrenaline.

Pseudohypoxic phenotype
Hypoxic-like metabolic changes that are observed in cells in the absence of a hypoxic environment or stimulant.

Neomorphic
A novel and/or non-canonical function of an enzyme.

Heterozygous germline mutations
Inheritance of one copy of a mutant allele and one copy of the wild type allele.

Loss of heterozygosity
(LOH). The loss of one allele of a genetic locus.

observed in multiple cancers including gliomas[72,104] and acute myeloid leukemia[105]. D-2-HG accumulation has also been demonstrated to occur as a result of loss-of-function mutations in *D2HGDH*, which are observed in a small subset of large B cell lymphomas[106]. In addition, off-target, substrate-promiscuous activity of D-3-phosphoglycerate dehydrogenase (PHGDH), which catalyses the conversion of αKG to D-2-HG, leads to accumulation of D-2-HG in *IDH*-wild type breast cancer cells[107]. These data implicate both increased synthesis of D-2-HG and its reduced conversion into αKG in D-2-HG accumulation in cancer.

According to the cBioPortal database, <1% of *IDH1* and *IDH2* mutations are found in large-scale cancer genomic studies of RCC such as the TCGA dataset[108,109]. Although substantial accumulations of 2HG were identified in human ccRCC tissues, >90% of this 2HG was the L-2-HG isoform[110], suggesting that D-2-HG is unlikely to have an important role in RCC pathogenesis. Reduced expression of L2HGDH was found to contribute to the accumulation of L-2-HG in patients with ccRCC[110]. LOH of the *L2HGDH* gene (which is located on chromosome 14q, a region that is commonly deleted in ccRCC)[76,111] correlated with reduced expression of L2HGDH and accumulation of L-2-HG, supporting the hypothesis that *L2HGDH* might function as a tumour suppressor in RCC[76]. Furthermore, among patients with ccRCC, loss of *L2HGDH* conferred a worse prognosis. Preliminary metabolomic profiling suggested that increased levels of L-2-HG might be associated with RCC tumour progression, which further corroborates its role as an oncometabolite[76].

Mutations in αKG dehydrogenase (α*KGDH*)[112], lipoic acid synthase (*LIAS*)[112] and lipoyltransferase-1 (*LIPT1*)[113] have also been implicated in 2HG accumulation. These genes encode enzymes that are required for the proper functioning of the αKGDH complex, which catalyses the conversion of αKG to succinyl-coenzyme A in the TCA cycle. These mutations result in a truncated TCA cycle that promotes the production of L-2-HG from the accumulated αKG[112,113]. Evidence suggests that the resulting downstream oncometabolite activity might inhibit PHDs, leading to HIF stabilization and HIF1-targeted activation of genes including VEGF and GLUT1 (REF. [112]). L-2-HG also accumulates and suppresses PHD activity and subsequent HIF activation in patients with homozygous germline mutations of enzymes of lipoic acid synthesis[112]. However, this rare inborn error of metabolism is generally lethal at a young age[112,114], which may preclude the development of oncometabolite-associated tumours in these patients. Characterization of patients with heterozygous germline mutations in *αKGDH* and *LIAS* may provide additional insight into the tumorigenic role of L-2-HG accumulation.

Exogenous origins of oncometabolites

Remarkably, oncometabolites have also been demonstrated to accumulate in cells and induce oncogenic transformation in the absence of oncogenic mutations[115]. Identifying the environmental factors linked to oncometabolite accumulation may shed light on how these factors have an impact on or predispose individuals to cancer.

A number of pathways for oncometabolite accumulation in response to exogenous stimuli have been identified. For example, hypoxia-induced, off-target, substrate-promiscuous activity of lactate dehydrogenase A (LDHA)[116] and malate dehydrogenase (MDH2)[116,117] on glutamine-derived αKG[23,116] results in L-2-HG accumulation in mammalian cells. In addition, acute ischaemic preconditioning in vivo resulted in accumulation of 2HG in mouse myocardium[118]. Although modest accumulations of L-2-HG in hypoxic cells were observed compared with those seen in cancer cells[23], hypoxia-induced L-2-HG accumulation was sufficient and necessary to exert recognized oncometabolite functions, such as repressive trimethylation of the histone protein, histone 3 lysine 9 (H3K9me3)[116]. Independent of hypoxia, acidic conditions have also been observed to drive L-2-HG accumulation by augmenting the promiscuous activity of LDH1 and MDH2, as well as inhibiting the activity of L2HGDH in vitro[119].

Succinate accumulation has also been reported in cancer cells grown under hypoxic conditions in a 3D tumour model[120] and in animal models subjected to ischaemia–reperfusion injury in vivo[121–123]. Succinate oxidation has been shown to contribute to cardiac injury at reperfusion[121,123,124] via the generation of reactive oxidative species (ROS)[121]. However, study of the chronic effects of succinate accumulation in these tissues, analogous to SDH-deficient tumours, is challenging because the accumulated succinate rapidly returns to baseline levels[121,123,124]. In the hypoxic retinas of rodents, succinate accumulation activates succinate receptor 1 (SUCNR1; also known as GPR91), which leads to the upregulation of angiogenic proteins such as VEGF in a HIF-independent manner[122]. Of note, the hypoxic induction of oncometabolite accumulation may be propagated and amplified by the oncometabolites themselves via stabilization of HIF expression[19,26,27,29,125,126]. In addition, succinate may participate in a positive feedback system that reinforces the *HIF1A* signalling loop. In lung adenocarcinoma cells, *HIF*-dependent expression of *miR-210* was demonstrated to target and downregulate *SDHD*[127]. The resulting succinate accumulation in turn led to HIF stabilization through inhibition of PHDs and thus perpetuated the hypoxic phenotype.

Mitochondrial dysfunction arising from glucose toxicity[128–130] can also result in oncometabolite production. Fumarate accumulation and fumarate-dependent protein succination were observed in the adipose tissues of hyperglycaemic mice[128,129], analogous to the protein succination that is observed in FH-deficient tumours[78,131] (discussed further below). A similar succination phenotype was observed in the adipose tissue of obese, insulin-resistant, non-hyperglycaemic mice[132], suggesting other potential exogenous sources of oncometabolite production. Evidence of succinate accumulation has also been found in bone marrow stromal cells of diabetic mice[133]. In these cells, succinate accumulation stimulated osteoclastogenesis via activation of Sucnr1. Of note, mitochondrial dysfunction has been linked to the metabolic syndrome, a distinct cluster of conditions including obesity, diabetes mellitus, hypertension, and hyperlipidaemia[134], which has been causally

linked to RCC[135]. Obesity, diabetes mellitus, hypertension and hyperlipidaemia are also associated with an increased risk of RCC[135,136]. This link provides a nidus for detailed investigations into the crosstalk between environment-induced oncometabolite accumulation and their tumorigenic role in RCC.

Infection has also been implicated in the exogenous production of oncometabolites. Lipopolysaccharide-induced activation of macrophages leads to succinate accumulation[137,138], downstream HIF stabilization and upregulation of the transcription of HIF-targeted genes such as *IL1B*, which encodes IL-1β (a key pro-inflammatory signalling molecule)[138]. Further elucidation of the mechanisms by which exogenous factors induce oncometabolite production independent of oncogenic mutations, the magnitude of these effects and the subsequent sequelae will be a crucial step forwards in understanding and ameliorating the role of these factors in tumorigenesis and tumour evolution.

Pro-oncogenic role of oncometabolites

Oncometabolites exhibit a multitude of downstream pro-oncogenic functions in RCC and other cancers. These include functions that converge on a group of downstream pro-oncogenic pathways and unique functions that are specific to individual oncometabolites (FIG. 2).

Common pro-oncogenic pathways

A characteristic trait that is shared by succinate, fumarate and 2HG is their ability to competitively inhibit αKG-dependent dioxygenases (αKGDDs)[67,139] owing to their structural similarity to αKG, which is an essential co-substrate for enzyme activity[71,140]. αKGDDs are a superfamily of enzymes that are involved in a plethora of biological processes. The most frequently studied αKGDDs with roles in oncometabolite signalling are PHDs, inhibition of which is involved in induction of the 'pseudohypoxic' milieu[27,47,67,68], the Jumonji C domain-containing histone lysine demethylases (KDM)[71,140–142], which have roles in histone demethylation, and the ten-eleven translocation (TET) enzyme family of 5-methylcytosine (5mC) hydroxylases, which are involved in DNA demethylation[71,140–143]. Inhibition of KDMs and TETS leads to hypermethylated phenotypes that are characteristic of tumour aggressiveness and metastatic progression.

Induction of a pseudohypoxic phenotype. As discussed above, the initial evidence supporting the concept of oncometabolites was the elucidation of the role of succinate and fumarate in inducing a pseudohypoxic milieu in SDH-deficient PGL and PCC, and in FH-deficient HLRCC, respectively[26,27,29,126]. As PHD hydroxylates HIF1α and HIF2α subunits and targets them for degradation, direct inhibition of PHD by fumarate or succinate ultimately culminates in aberrant HIF stabilization with downstream upregulation of HIF-targeted genes such as VEGF and GLUT1 (REFS[19,26,27]). Notably, SDH-deficient and FH-deficient tumours tend to exhibit intense vascularization and hypoxic gene signatures in keeping with a pseudohypoxic tumour phenotype[51–55]. Interestingly, HIF1α and/or HIF2α inactivation in SDHB-deficient osteosarcoma cells significantly impaired tumour growth in a mouse xenograft model[144], whereas HIF1α or HIF2α inactivation in a FH-deficient mouse model of renal cyst disease exacerbated or failed to ameliorate this phenotype, respectively[145]. These studies highlight the complex role of HIF and pseudohypoxia in tumorigenesis and suggest that this role might be context dependent (for example, cell-type specific)[67].

L-2-HG has also been shown to inactivate PHDs and aberrantly stabilize HIF1α[112,146,147], whereas the effect of D-2-HG on PHD remains contentious. Unexpected agonistic activity of D-2-HG on PHDs has been observed in vitro[112,147]. This finding has been challenged, however, owing to the observation of non-enzymatic oxidation of D-2-HG to form αKG in vitro[148]. This process would provide the co-substrate (αKG) that is necessary for PHD activation.

Epigenetic dysregulation. Oncometabolites have a role in epigenetic alterations through direct inhibition of KDMs[141,142] and TETs[143]. The resulting hypermethylation phenotypes alter the expression of a wide range of genes involved in cellular differentiation and the acquisition of malignant features. The epigenetic effects of DNA and histone methylation on transcriptional activity are challenging to distinguish as they are often interdependent and inter-regulated[149]. In general, histone hypermethylation (for example, owing to KDM inhibition) results in either transcriptional gene repression or activation, depending on the type of histone residues and the number of methyl groups added[67,149]. DNA methylation at CpG islands usually represses downstream gene transcription[67,149]. Oxidation by TET enzymes converts 5mC to hydroxylated 5mC (5hmC)[67,149] and primes the cytosine for demethylation, which generates unmethylated cytosine (5C). Global DNA hypomethylation, which leads to inappropriate transcriptional activity and chromosomal instability, coupled with specific patterns of hypermethylated CpG promoter islands, particularly upstream of tumour suppressor genes, which represses their expression, is characteristic of many tumour types[149–151].

Several studies have investigated the oncometabolic effects of histone and DNA hypermethylation owing to inhibition of KDM and TETs in *FH*-mutant, *SDH*-mutant and *IDH*-mutant tumours[71,140–142,146,152–154]. SDH-deficient tissues from patients with PGL and PCC demonstrated high levels of repressive histone marks (trimethylation of histone H3 at lysine 4 (H3K27me3))[141]. In addition, succinate accumulation in *SDHB*-knockout chromaffin cells[141] and *SDHB*-knockdown murine ovarian cancer cells[155], as well as 2HG accumulation in *IDH1*-mutant murine fibroblasts and *IDH1*-mutant glioma cells[71,142], led to inhibition of KDM and TET with characteristic hypermethylation phenotypes associated with suppression of cellular differentiation[141,142] and activation of epithelial-to-mesenchymal transition (EMT; a hallmark of tumour aggressiveness and metastatic progression) owing to upregulation of positive or downregulation of negative EMT regulator genes[141,155]. HLRCC-derived FH-deficient cells also elicited an EMT signature via fumarate-induced TET-mediated epigenetic suppression of miR-200, which has tumour-suppressive

CpG islands
Clusters of dinucleotide sequence of a cytosine followed by a guanosine nucleotide in the 5′–3′ direction. CpG islands are often found in promoter regions upstream of transcription sites.

effects on EMT gene expression[77]. The EMT phenotypic switch induced by oncometabolite accumulation in *FH*-mutant and *SDH*-mutant RCC tumours no doubt contributes to their clinically aggressive behaviour. In vivo, silencing of *SDHA* or *FH* in mouse hepatocytes led to the accumulation of succinate or fumarate, respectively, with evidence of KDM and TET inhibition and regulation of target gene expression[140]. DNA hypermethylation associated with repression of genes with roles in cell differentiation has been observed at CpG island sites in patients with *IDH1* and *IDH2* mutant chondrosarcoma[153] and more globally in acute myeloid leukaemia[152]. Furthermore, accumulations of D-2-HG resulting in increased DNA methylation (5mC) with concurrent decreased DNA hydroxymethylation, indicating TET inhibition, were observed in human *IDH1* glioma tissue[71] and in various cell types with ectopic expression of mutant *IDH1* or *IDH2*, leading to a block in cellular differentiation[152–154]. A lack of cell differentiation is a hallmark of malignancy and cancer progression[156,157] and a

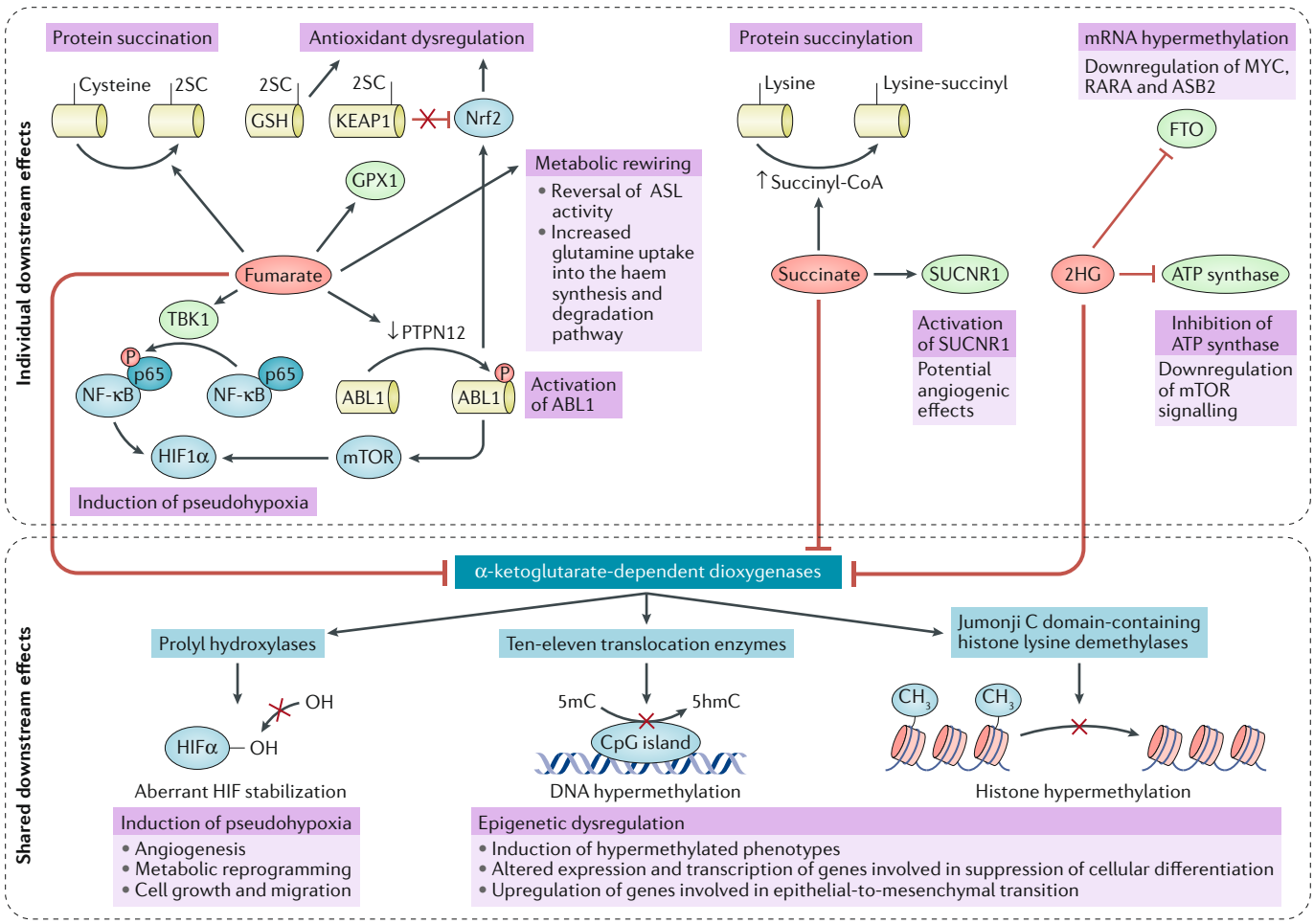

Fig. 2 | **Individual and shared oncometabolite signalling pathways.** The oncometabolites fumarate, succinate and 2-hydroxyglutarate (2HG) have individual and shared downstream effects. Fumarate accumulation can lead to antioxidant dysregulation in cells through succination of Kelch-like ECH-associated protein-1 (KEAP1), which removes its repressive effects on nuclear factor erythroid 2-related factor (Nrf2), thus leading to the upregulation of antioxidant genes[145,163], and of glutathione (GSH), which depletes the antioxidant capacity of the cells and renders them susceptible to accumulation of reactive oxygen species (ROS)[169]. Fumarate can also bind directly to glutathione peroxidase 1 (GPX1), a ROS scavenging enzyme[174], and can activate the Nrf2 signalling pathway via activation of tyrosine-protein kinase ABL1 (REF.[177]), which confers a proliferation advantage. In addition, fumarate can induce a pseudohypoxic phenotype through activation of tank-binding kinase 1 (TBK1), which leads to nuclear factor-κB (NF-κB) activation and subsequent transcription of hypoxia-inducible factor 1α (HIF1α)[165,166] as well as through activation of ABL1, which activates the mTOR–HIF1α pathway[164]. Fumarate also modulates cellular metabolism via reversal of the activity of the urea cycle enzyme arginosuccinate lyase (ASL)[102], as well as by diverting increased glutamine uptake into haem-associated pathways to sustain mitochondrial NADH levels and mitochondrial membrane potential[179]. Succinate activates the succinate receptor 1 (SUCNR1), which has a range of potential functions, including angiogenic effects[122,180], and is associated with succinylation of various proteins including histones[187,188,190]. The D-2-hydroxyglutarate isoform of 2HG exerts an antitumour effect via inhibition of the α-ketoglutarate-dependent dioxygenase FTO, which leads to mRNA hypermethylation and downregulation of genes associated with cell growth and transformation[195], as well as via direct inhibition of ATP synthase with subsequent downregulation of mTOR signalling[198]. The shared downstream effects of fumarate, succinate and 2HG converge on inhibition of α-ketoglutarate-dependent dioxygenases. This inhibition leads to epigenetic dysregulation through inhibition of ten-eleven translocation enzymes and Jumonji C domain-containing histone lysine demethylases, as well as induction of pseudohypoxia via inhibition of prolyl hydroxylases and resulting aberrant stabilization of HIFs[27,71,140–143]. The resulting hypermethylation and pseudohypoxic phenotypes are associated with tumour progression and aggressive disease. 2SC, S-(2-succino)-cysteine; 5hmC, 5-hydoxymethylcytosine; 5mC, 5-methylcytosine.

block in differentiation is thought to enable cancer cells to retain their ability to proliferate and propagate mutant clones, contributing to tumorigenesis[156,157].

Identification of specific DNA hypermethylation patterns within a subset of colorectal cancers[158] gave rise to the CpG island methylator phenotype (CIMP)-associated cancer subtypes, which are characterized by their extensive epigenomic aberrations and distinct biology[154,158,159]. CIMP subtypes have been increasingly recognized in other malignancies including gliomas (G-CIMP)[154,159,160] and in a subset of type 2 papillary RCC (CIMP-RCC)[5,161]. G-CIMP tumours are tightly associated with *IDH1* mutations[154,160] and the introduction of mutant IDH1 into human primary astrocytes led to accumulation of D-2-HG, inhibition of TET and a DNA hypermethylation profile that mirrored the changes observed in G-CIMP[154]. CIMP-RCC confers the worst prognosis of all the RCC subtypes and has been associated with early-onset disease and, perhaps unsurprisingly, germline or somatic mutations of the *FH* gene[5,161]. Given the role of oncometabolites in αKGDD inhibition, including inhibition of TET enzymes, it is plausible that fumarate accumulation may be causally linked to the hypermethylated state in CIMP-RCC. FH-deficient RCC is highly aggressive[94,95,162] and current therapies are ineffective in treating advanced FH-deficient RCC[163,164]. Further understanding of the underlying disease process might enable the development of more effective therapies for CIMP-RCC and FH-deficient RCC. Such strategies might include the use of histone and DNA methylation inhibitors, which are discussed further below.

Several studies have demonstrated that oncometabolites have varying half maximal inhibitory concentration (IC_{50}) values for different αKGDDs[71,140,143,146], suggesting that oncometabolite type and accumulation levels might determine the precise nature of downstream oncogenic processes in individual cell types. Beyond the common inhibition of αKGDDs, the distinct biological functions of individual metabolites are now beginning to be appreciated.

Individual oncometabolite functions

Fumarate. To date, fumarate has been demonstrated to be the most versatile of the bona fide oncometabolites, having an impact on oncogenic signalling, antioxidant response and phenotype switching (FIG. 2). In addition to the direct inhibition of PHD enzymes that facilitates induction of pseudohypoxia, fumarate has been shown to drive a hypoxic phenotype on a transcriptional level through the non-canonical activation of nuclear factor-κB (NF-κB)[165], a family of transcription factors that can promote HIF1α transcription[166]. This signalling pathway is dependent on fumarate activation of tank-binding kinase 1 (TBK1), an enzyme that phosphorylates p65 (a subunit of NF-κB) with subsequent NF-κB activation[165]. Inhibition of the TBK1–p65 axis in FH-deficient RCC cells blocked HIF1α expression and reduced cellular invasion in vitro, suggesting a novel treatment target[165]. Similarly, silencing of HIF1α in HLRCC-derived, FH-deficient RCC cell lines diminished the invasive properties of these cells[167]. These findings support a critical tumorigenic role of HIF1α and pseudohypoxia in aggressive RCC subtypes such as FH-deficient RCC (HLRCC)[16,56,168]. Potentially contradicting this theory, genetic inactivation of HIF1α or HIF2α in FH1-deficient mice exacerbated or failed to ameliorate the renal cyst phenotype, respectively, suggesting alternative mechanisms for oncogenesis in FH-deficient cells[145].

An alternative candidate oncogenic pathway in FH-deficient disease via stabilization of the nuclear factor erythroid 2-related factor (Nrf2) antioxidant pathway has been proposed[145,163]. As mentioned above, a distinct feature of FH-deficient tumours is the ability of accumulated fumarate to modify a wide range of proteins via succination[78,131]. This post-translational modification can impair protein function, and is caused by fumarate reacting with specific cysteine residues on proteins, producing *S*-(2-succino)-cysteine (2SC) residues[78,131]. A key target of succination is Kelch-like ECH-associated protein-1 (KEAP1). Succination of KEAP1 abrogates its repressive effects on Nrf2, resulting in the upregulation of Nrf2-dependent genes, which are involved in antioxidant pathways that increase the ability of cells to adapt to oxidative stress[145,163]. In keeping with this finding, Nrf2 and downstream Nrf2-targeted genes were upregulated in HLRCC-derived type 2 pRCC tumour cells[145], suggesting potential novel therapeutic targets for this aggressive disease[145,163]. By contrast, succination of the antioxidant glutathione in FH-deficient RCC cells depletes their antioxidant capacity, rendering them susceptible to endogenous accumulation of ROS[169], subsequent stabilization of HIF1α[169] and induction of cellular senescence[170]. This state of irreversible growth arrest is linked to activation of tumour suppressor pathways such as p53 and retinoblastoma, and is thought to protect against cancer[171,172]. Ablation of a key mediator of senescence, p21, in FH1-deficient mice induced the transformation of benign renal cysts into hyperplastic lesions, suggesting that this fumarate-induced senescent event needs to be overcome for renal tumorigenesis to proceed[170]. Although ROS alone can activate the Nrf2 signalling pathway through KEAP1 inhibition[173], fumarate-dependent succination of KEAP1 seems to be the predominant mechanism for Nrf2 activation in FH-deficient cells[169,170].

Although not demonstrated in the context of FH-deficiency, fumarate has been found to bind directly to glutathione peroxidase 1 (GPX1) and activate this ROS scavenging enzyme in breast and lung cancer cells, conferring a proliferative advantage by regulating redox homeostasis[174]. Glutamate dehydrogenase 1 (GDH1), which converts glutamate to αKG, was identified to control the intracellular levels of αKG (which is subsequently converted to fumarate) in these cells[174]. Furthermore, GDH1 inhibition attenuated cancer cell proliferation and tumour growth in vivo[174]. Of note, GDH1 expression was upregulated in human breast and lung cancer tissues and correlated with disease progression[174]. Given the fumarate accumulation that occurs in FH-deficient tumours and the finding that glutamine entry into the TCA cycle (via GDH1) is a dominant pathway in this setting[175,176], it is plausible that GDH1 inhibition might also have antitumoural effects in the setting of FH-deficiency. Overall, the available data in

Half maximal inhibitory concentration (IC_{50}). A measure of the potency of a substance to inhibit a specific biological process or function by 50%.

FH-deficient cells indicate that they have highly adapted and intrinsic mechanisms that combat redox stress in a multi-layered approach that promotes tumour survival.

Identification of *ABL1*, which encodes the tyrosine-protein kinase ABL1 and is upregulated in FH-deficient tumours[164], connected the HIF and Nrf2 pathways. Fumarate-mediated activation of ABL1 occurs via oxidative-stress-induced suppression of tyrosine-protein phosphatase non-receptor type 12 (PTPN12)[177], which leads to activation of the Nrf2 antioxidant pathway and the mTOR–HIF1α signalling pathway in FH-deficient RCC cells[164]. Furthermore, ABL1 inhibition suppressed the invasion capacity and growth of these cells in vitro and in vivo[164,177]. As ABL1 acts upstream of two major pathways (HIF and Nrf2) that have been implicated in FH-deficient tumours, this finding suggests that targeting ABL1 might affect multiple pathways that are essential for these tumours.

Another unique oncometabolic feature of fumarate is its ability to directly modulate cellular metabolism. In normal cells, fumarate participates in several major interlinked pathways such as the TCA cycle and the urea cycle[102,178]. Accumulation of fumarate in FH-deficient cells has been shown to reverse the activity of the urea cycle enzyme ASL[102]. Normally, argininosuccinate is produced from citrulline and aspartate via argininosuccinate synthetase in the urea cycle and is converted into fumarate and arginine via ASL. Reversal of ASL activity results in an accumulation of argininosuccinate and renders FH-deficient cells auxotrophic for arginine[102]. As expected, arginine depletion in these cells led to reduced cellular survival and proliferation in vitro[102]. The loss of FH also leads to a complex metabolic rewiring pattern involving the diversion of increased glutamine uptake into the haem synthesis and degradation pathway, which critically sustains mitochondrial NADH levels and mitochondrial membrane potential[179]. Targeting this unique FH-deficient haem pathway, in particular via the inhibition of haem oxygenase 1, which catalyses the degradation of haem, rendered a selective synthetic lethality to FH-deficient cells, which spared normal tissues with wild type FH[179]. These two studies[102,179] highlight how FH-specific liabilities can be manipulated to provide novel strategies to treat FH-deficient tumours such as those that occur in patients with HLRCC.

Perhaps unsurprisingly, given the multitude of pro-oncogenic pathways involving fumarate, increased gene expression of *FH* correlates with better survival outcomes in RCC[10], whereas *FH* gene suppression correlates with a very poor prognosis[5,77,161]. Furthermore, FH is suppressed in a large subset of patients with ccRCC and this suppression correlates with EMT and poor prognosis[77]. Therefore, identifying the pervasive sequelae of fumarate accumulation in these tumours could lead to the development of more effective and targeted therapies for the management of FH-deficient RCC.

Succinate. In addition to its inhibitory effect on αKGDDs, succinate exhibits distinct oncometabolite features that may have an impact on the phenotype of SDH-deficient tumours. Activation of Sucnr1 by high levels of succinate has been shown to upregulate angiogenic proteins, including VEGF, in a HIF-independent manner in rodent hypoxic retinal ganglion cells[122], as well as to induce an angiogenic phenotype in human endothelial cells in vitro and in transgenic zebrafish in vivo[122,180]. Activation of this succinate–SUCNR1 signalling axis may also be an important pathway in tumour angiogenesis[122,180] and demonstrates the ability of succinate to exhibit hormone-like traits. Elevated circulating levels of succinate that activate the Sucnr1 pathway have also been implicated in renovascular hypertension via activation of the renin–angiotensin system in kidneys[181,182]. Hyperglycaemia also triggers the succinate–Sucnr1 pathway, potentially implicating it in the pathophysiology of diabetic nephropathy[183]. The succinate–Sucnr1 signalling axis has also been implicated in the pathological hypertrophy of ischaemic cardiomyocytes[184] and in the activation of fibrosis in ischaemia-induced liver damage[185].

SDH-deficiency has been associated with the post-translational protein modification known as succinylation, which differs from fumarate-induced succination[186–188]. Succinylation results from a reaction of succinyl-CoA with the lysine residues in proteins[187,188]. The mechanism that underlies the link between SDH deficiency and succinylation is thought to be an increase in the levels of succinate, which can equilibrate with succinyl-CoA[187]. Interestingly, D-2-HG accumulation competitively inhibits its SDH activity in *IDH1*-mutant fibrosarcoma cells, causing a hypersuccinylated phenotype that induces changes in metabolism and apoptosis resistance[189], which are established hallmarks of cancer[24]. Either re-expression of the desuccinylase SIRT5 or glycine supplementation led to reversal of this hypersuccinylated phenotype and slowed oncogenic growth in vitro[189]. Mechanistically, glycine depletes the availability of succinyl-CoA by condensing directly with succinyl-CoA to form 5-aminoevuilinc acid, which enters the haem biosynthesis pathway[189]. Remarkably, type 2 pRCC tumours with *FH*-mutations were found to be hypersuccinylated compared with *FH*-wild type RCC[189], demonstrating the likely convergence of oncometabolites in this process. In addition, several key metabolic enzymes such as MDH and IDH2, as well as histones[190], are targets of protein succinylation[187,191], possibly suggesting an autoregulatory role in metabolism and perturbation of the cellular epigenome; however, these functional effects are yet to be fully elucidated[138,191,192].

Lastly, SDH-deficient cells have been reported to depend on pyruvate carboxylase (PC) to funnel pyruvate into the truncated TCA cycle for biosynthesis of aspartate, which is an important precursor for sustaining cellular growth[193,194]. Silencing PC expression attenuated SDH-deficient tumour growth in vivo in a mouse model[193]. This finding, together with the increased mRNA expression of *PC* in a range of human SDH-deficient tumours and PC protein expression in SDH-deficient RCC, suggests that PC might be a potential target for synthetic lethality in SDH-deficient RCC[193].

As mentioned above, SDH-deficient RCC is a rare cancer that can exhibit an aggressive phenotype with an early age of onset[85,89]. Elucidating the common pathways and individual sequelae of succinate accumulation in the

setting of RCC will form the basis for future strategies for the management of this challenging disease.

2-Hydroxyglutarate. The discovery and elucidation of the mechanisms of L-2-HG accumulation in ccRCC[76,110] highlights the importance of the tumorigenic role of this oncometabolite. As discussed above, 2HG exists in two isoforms (L-2-HG and D-2-HG), which are produced by distinct biological mechanisms that are differentially upregulated in cancers and exhibit distinct features beyond αKGDD enzyme inhibition[60,68].

Interestingly, studies of 2HG in leukaemic cells have yielded conflicting results. In multiple *IDH*-wild type leukaemic cell lines, addition of exogenous D-2-HG resulted in dose-dependent inhibition of cell proliferation and viability[195]. By contrast, another study reported that the addition of exogenous D-2-HG at a comparable concentration in similar *IDH*-wild type cell lines resulted in a contrasting phenotype of cell proliferation and leukaemic transformation[115]. This disparity has been partly attributed to the differing in vitro conditions used in these experiments[195]. However, in support of an antitumoural effect, accumulation of D-2-HG (owing to either exogenous addition or *IDH* mutation) attenuated disease progression and increased survival in an in vivo xenograft model of leukaemia[195]. Mechanistically, D-2-HG competitively inhibits the mRNA demethylase α-ketoglutarate-dependent dioxygenase FTO[195], which is a member of the αKGDD family[195,196]. FTO in turn downregulates the expression of target genes such as *MYC*, *RARA* and *ASB2*, which have roles in promoting cell growth and transformation[195,197]. Interestingly, these findings were recapitulated in *IDH*-wild type glioma cells exposed to D-2-HG and in human leukaemia cells exposed to exogenous L-2-HG[195], suggesting a convergence in the function and phenotypic effects of these 2HG isoforms across multiple cancers. Furthermore, direct inhibition of ATP synthase and subsequent downregulation of mTOR signalling by D-2-HG accumulation in *IDH1*-mutant glioma cells in vitro and in vivo suggests that D-2-HG might have growth-suppressive functions and further corroborates its antitumoural effects[198]. This phenomenon may partially explain the correlation between *IDH*-mutations in gliomas and improved patient prognosis[198,199].

One explanation for the convincing simultaneous pro-tumoural and antitumoural roles of 2HG is that its effects are contingent on the specific cancer and/or specific stage in tumour evolution (that is, tumour initiation versus tumour progression)[195,198]. Supporting this notion, accumulations of D-2-HG and L-2-HG were observed in colorectal cancer cell lines in the absence of *IDH*, *D2HGDH* or *L2HGDH* mutations[200]. Dissecting the individual roles of the 2HG isoforms in this setting revealed that D-2-HG, but not L-2-HG, has pro-tumoural roles in EMT gene upregulation through KDM inhibition and subsequent histone hypermethylation, as well as in the acquisition of an invasive and migratory cell phenotype[200]. This phenotype was ameliorated by the addition of a glutaminase inhibitor[200], indicating that D-2-HG and L-2-HG accumulation in colorectal cancer cells is dependent on glutamine-derived anaplerosis. In keeping with these findings, elevated D-2-HG levels in colorectal cancer samples correlated positively with higher frequencies of distant metastases[200]. Ascertaining the IC_{50} levels for D-2-HG and L-2-HG in colorectal cancer may provide an insight into the differing effects of these oncometabolites[67,71,140,143,146].

Overall, the available studies indicate that both 2HG isoforms can converge on a range of non-metabolic functions, such as DNA and histone hypermethylation[71,76,142,146,152], but potentially diverge in respect to their pro-tumoural and antitumoural effects, as well as in the 2HG isoform implicated in different types of cancer[76,112,115,119,142,195,197,198,200]. Thus, it is imperative that future studies that aim to elucidate the role of 2HG isoforms in tumours and the associated potential clinical applications are cancer- and tumour-stage specific.

Targeting oncometabolites

Multiple potential targets for therapeutic intervention within oncometabolite-associated pathways have been identified (FIG. 3). These approaches can broadly be divided into targeting oncometabolite accumulation (that is, production and/or degradation pathways) or targeting the downstream sequelae either broadly (for example, using DNA hypomethylation agents)[153] or targeting specific pathways (for example, arginine deprivation in FH-deficient RCC)[102,201] (TABLE 1).

Oncometabolite accumulation

Targeting the pathways that contribute to oncometabolite accumulation has shown promising results with evidence of translation into clinical practice[202]. One such example is the development of specific mutant-IDH1 and mutant-IDH2 inhibitors that reduced D-2-HG levels and reversed the DNA and histone hypermethylation profile and cellular differentiation block in leukaemia in preclinical studies[115,203–205]. This effort, together with initial clinical studies that demonstrated a promising overall response rate in patients with *IDH1*-mutant and *IDH2*-mutant relapsed or refractory AML[202,206], resulted in the approval of ivosidenib in 2018 and enasidenib in 2017, for use in the management of these patients[202,207]. As mutant IDH has not been implicated in the pathophysiology of oncometabolite-associated RCC, these inhibitors are unlikely to be beneficial in this setting.

Another rapidly emerging area of interest is the use of glutaminase inhibitors in oncology. Cancer cells have long been recognized to rely on glutamine as an essential fuel source and biosynthetic precursor to support the demands of rapid growth, survival and cellular stress[175]. Glutamine has several fates; however, the conversion of glutamine to αKG as an anaplerotic source for the TCA cycle is of particular relevance in cancer. The first step in this process — the conversion of glutamine into glutamate — is catalysed by glutaminase[175]. Cancers with defective mitochondria, such as FH-deficient and SDH-deficient RCC, predominantly utilize glutamine-derived αKG for reductive carboxylation (FIG. 1), enabling these cells to bypass the truncated TCA cycle and replenish essential TCA cycle intermediates such as citrate, which is cleaved to form acetyl-coenzyme A for lipid biosynthesis[175,176]. Glutamine-derived αKG

Anaplerosis
The process of replenishing the tricarboxylic acid cycle intermediates that have been extracted for biosynthesis.

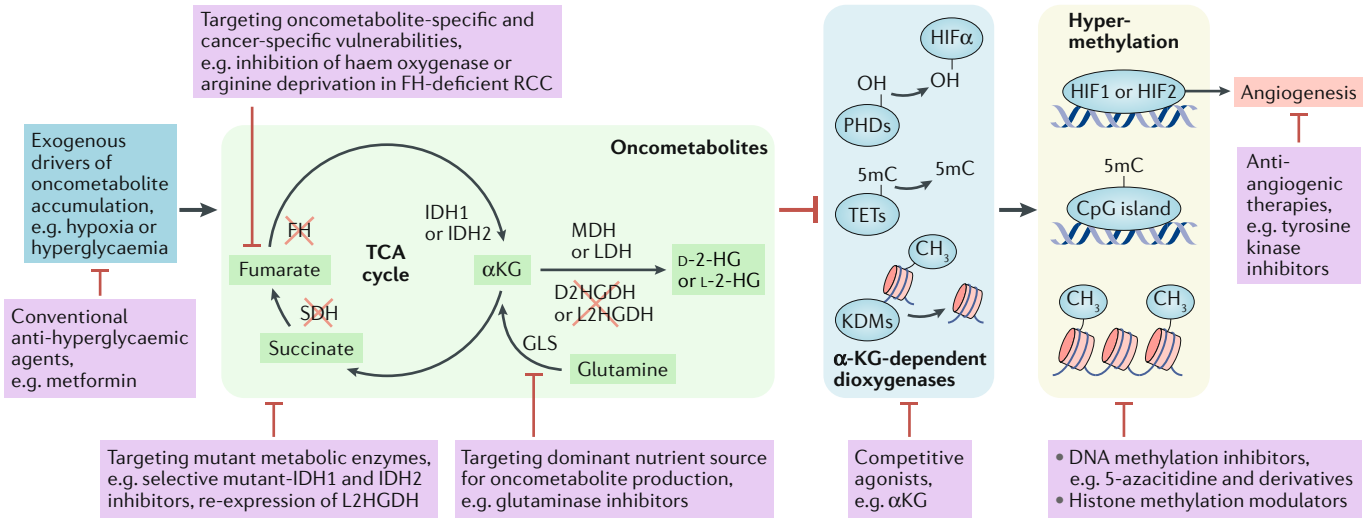

Fig. 3 | **Strategies for targeting oncometabolite-associated pathways.** Potential strategies for the therapeutic targeting of oncometabolites range from targeting the exogenous drivers of oncometabolite production, to the nutrient sources and enzymatic perturbations involved in the accumulation of oncometabolites and the downstream enzymatic, epigenetic and phenotypic effects of oncometabolite accumulation. Elucidating the mechanisms that underlie specific pathways of oncometabolite production and their downstream sequelae will enable a more informed choice of potential therapeutic strategies for individual oncometabolite-associated tumours. Opportunities also exist for multimodal or multi-layered synergistic approaches. 5mC, 5-methylcytosine; αKG, α-ketoglutarate; CpG, cytosine-guanosine dinucleotide; D-2-HG, D-2-hydroxyglutarate; D2HGDH, D-2-HG dehydrogenase; FH, fumarate hydratase; GLS, glutaminase; HIF, hypoxia-inducible factor; IDH, isocitrate dehydrogenase; KDM, histone lysine demethylase; L-2-HG, L-2-hydroxyglutarate; L2HGDH, L-2-HG dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; PHD, prolyl hydroxylase; RCC, renal cell carcinoma; SDH, succinate dehydrogenase; TET, ten-eleven translocation.

also seems to be the dominant source for 2HG production in several cancer types, including breast cancer[208], chondrosarcoma[209], colorectal cancer[200] and RCC[76], as well as the main source of fumarate in FH-deficient RCC cells[175,176]. As discussed above, substantial accumulation of L-2-HG occurs in human ccRCC tissues[110] owing to LOH of the *L2HGDH* gene[110] and the promiscuous activity of MDH2 on predominantly glutamine-derived αKG[76]. Targeting the 'production' pathway of 2HG accumulation, the glutamine–MDH2 axis, via pharmacological or genetic inhibition significantly reduced L-2-HG levels and suppressed the migratory phenotype in multiple RCC cell lines with restoration of epigenetic TET activity, as demonstrated by elevated DNA 5hmC levels in vitro[76]. Moreover, glutaminase inhibition in vivo suppressed RCC tumour growth[76], adding to the evidence that targeting glutamine in this setting profoundly affects L-2-HG accumulation, with suppression of tumour phenotype.

Several other independent studies that investigated glutaminase inhibition in the wider context of RCC, including in *VHL*-mutant and *VHL*-wild type RCC, also demonstrated suppression of tumour growth in vivo[14,210,211]. These studies facilitated the translation of glutaminase inhibitors into several phase 1/2 clinical studies, either as a monotherapy or in combination with approved therapeutic agents[212–214]. The study cohorts included patients with FH-deficient and SDH-deficient RCC, as well as patients with metastatic RCC. Early results are promising, with an overall response rate of 40% and a favourable safety profile in patients with advanced and/or metastatic ccRCC[212,214].

Not all RCC tumours accumulate L-2-HG; therefore, it is highly unlikely that the effects of glutaminase inhibition in RCC are purely mediated through this metabolite. As loss of *L2HGDH* and accumulation of L-2-HG confers worse prognosis in patients with ccRCC[76], and upregulation of the glutamine transporter also correlates with aggressive ccRCC and a worse prognosis[10], it would be of immense value to ascertain whether there is crosstalk between these underlying mechanisms that confer a poor prognosis. Given the ability of L-2-HG accumulation to significantly modulate the epigenetic cell state (and thereby lead to an aggressive tumour phenotype), it may be plausible that upregulation of glutamine transporters leads to increased uptake of glutamine and conversion of glutamine-derived αKG to L-2-HG, which might underlie the correlation of glutamine transporter upregulation with aggressive ccRCCs. Determining whether glutaminase inhibition has a more profound effect in L-2-HG-associated RCC tumours given the pro-oncogenic capabilities of this oncometabolite would also be of value. In addition to inhibition of the glutamine–MDH2 axis, genetic restoration of *L2HGDH* suppressed RCC tumour growth in vivo[76]. These findings highlight several vulnerabilities in both the production and degradation pathways of L-2-HG that can be exploited for the development of targeted therapies in L-2-HG-associated RCC. Establishing whether the use of multiple approaches to reduce L-2-HG levels has a synergistic effect may have an impact on the strategic management of this subset of RCC tumours. Unlike MDH2, L-2-HG has no known physiological role[59–62]; therefore, specific targeting of L2HGDH may be preferable over targeting of MDH2, which could potentially lead to undesirable systemic effects.

As discussed above, increasing evidence supports a role of exogenous factors in oncometabolite production.

Table 1 | **Oncometabolite-associated RCC subtypes, clinical features and potential therapeutic strategies**

Oncometabolite (mutated genes)	Associated RCC subtype(s) and clinical features	Potential therapeutic strategies	Refs
Fumarate (*FH*)	HLRCC-associated RCC; 14–18% of patients with HLRCC will develop pRCC type 2; early onset (mean age 10–44 years); highly aggressive phenotype and early metastasis; bilateral; mainly pRCC type 2, but also described as solid, tubulocystic, cribriform or cystic	Arginine deprivation; haem oxygenase inhibition; ABL1 inactivation; targeting TBK1–p65 axis; GDH1 inhibition; glutaminase inhibition; DNMT inhibition; exogenous αKG; HIF2α inhibition	4,16,26, 93,94,102, 162,174,179, 201,247
Succinate (*SDHA; SDHB* (82%); *SDHC; SDHD; SDHAF2*)	SDH-deficient RCC; accounts for 0.2% of all RCC; early onset (mean age 37–46 years); aggressive phenotype; bilateral in 26% of patients; associated with paraganglioma (25% of patients)	SIRT expression; exogenous glycine; PC inhibition; DNMT inhibition; glutaminase inhibition; exogenous αKG; HIF2α inhibition	4,83,84,86–90, 97,189,191, 193,248–250
L-2-hydroxyglutarate (*L2HGDH*)	ccRCC; most common RCC subtype (~70–80%); mean age of onset 64 years; aggressive phenotype; lower L2HGDH expression and increased L-2-HG levels are associated with tumour progression and/or worse survival; associated with Wilms tumour	L2HGDH re-expression; MDH2 inhibition; DNMT inhibition; glutaminase inhibition; exogenous αKG; HIF2α inhibition	65,76,110, 251,252

αKG, α-ketoglutarate; ccRCC, clear cell RCC; DNMT, DNA methyltransferase; FH, fumarate hydratase; GDH, glutamate dehydrogenase; HIF2α, hypoxia-inducible factor 2α ; HLRCC, hereditary leiomyomatosis and renal cell cancer; L-2-HG, L-2-hydroxyglutarate; L2HGDH, L-2-HG dehydrogenase; MDH2, malate dehydrogenase 2; PC, pyruvate carboxylase; pRCC, papillary RCC; RCC, renal cell carcinoma; SDH, succinate dehydrogenase; SDHAF, succinate dehydrogenase assembly factor; SIRT, sirtuin; TKB1, TANK-binding kinase 1.

Targeting the modifiable exogenous factors that have been implicated in oncometabolite accumulation may ameliorate their pro-tumoural effects. Although the mechanisms have not been fully elucidated, compelling evidence suggests that hyperglycaemia-induced oncometabolite production elicits cell phenotypes that are analogous to those observed in oncometabolite-associated cancers[78,128,129,131]. Thus, studying the role of antidiabetic therapies such as metformin may also be of interest in oncometabolite-associated tumours. Of note, metformin is currently in oncological clinical trials in patients with breast and prostate cancer. Although the available data are conflicting, metformin has been shown to have a degree of antitumoural activity and has also been demonstrated to modulate numerous metabolic pathways[40,215].

Downstream oncometabolite sequelae

Potential therapeutic strategies have been developed to tackle broad oncometabolite-induced pathways, as well as oncometabolite-associated phenomena observed in specific cancer types (FIG. 3). Here, we focus on the common downstream oncometabolite-induced pathways with reference to RCC where applicable. Potential strategies to target individual oncometabolite phenomena are discussed in the individual oncometabolite sections above (TABLE 1).

The general convergence of oncometabolites on the inhibition of αKGDD enzymes led to an early and straightforward strategy of overcoming competitive inhibition by administering αKG in excess. Studies in SDH-deficient cancer cells and in RCC cells treated with exogenous fumarate showed that administration of αKG led to a reversal of the HIF pseudohypoxic drive through restoration of PHD activity[216,217], as well as reversal of DNA 5mC accumulation, indicative of restoration of TET activity[141]. Dose-dependent suppression of HIF1α and VEGF protein levels by αKG were also observed in lung cancer cells and in hepatocellular carcinoma cells in vitro[218,219]. Furthermore, addition of αKG to human colorectal cancer cells under hypoxic conditions led to PHD-induced destabilisation of HIF expression and PHD-dependent hypoxic cell death[220]. Consistent with these findings, treatment with αKG had antitumoural effects in vivo, suppressing tumour growth and angiogenesis in a lung cancer xenograft model[219]. These preclinical studies suggest that utilizing αKG could meaningfully reverse oncometabolite-induced αKGDD inhibition at a molecular and phenotypic level in a wide variety of cancer subtypes. As epigenetic dysregulation and pseudohypoxia drive are strongly implicated in the pathogenesis and progression of RCC, the broad targeting of αKGDD combating both these elements warrants further investigation.

Targeting HIF transcription factors may be another promising therapeutic approach. Attenuation of tumour growth upon HIF2α inhibition was demonstrated in vitro and in multiple RCC tumour graft models[221,222]. These studies helped to lay the foundation for the first human studies and clinical trials of HIF2α inhibition in patients with locally advanced and metastatic ccRCC. Promising early results demonstrated complete responses, partial responses or stable disease in two-thirds of these patients[221,223]. Given the robust capability of oncometabolites to induce the HIF-signalling pathway independent of VHL-deficient RCC, these studies might lead to improved management strategies for rare and aggressive oncometabolite-associated RCCs.

Treatment with DNA methyltransferase (DNMT) inhibitors (also known as DNA hypomethylation agents) such as 5-azacitidine, has been shown to improve outcomes and delay transformation in patients with high-risk myelodysplastic syndrome[224,225]. DNMT inhibitors have also demonstrated potential in ameliorating hypermethylation phenotypes in oncometabolite-associated tumours[141,153,226]. Studies using low doses of DNMT inhibitors demonstrated impairment in cell growth, reversal of the migratory phenotype and restoration of cell differentiation in a range of *SDH*-knockout, *IDH1*-mutant and *IDH2*-mutant cell lines[141,153,226]. Furthermore, the DNMT inhibitor decitabine (a derivative of 5-azacitidine) reversed DNA methylation marks in *IDH1*-mutant glioma cells in vitro and suppressed the growth of *IDH1*-mutant glioma in a mouse model[226]. These studies provide a potential strategy for targeting oncometabolite-induced DNA-related epigenetic modifications; however, histone methylation also has a role in modulating transcriptional activity.

Simultaneous targeting of multiple epigenetic modifiers may therefore prove to be more efficacious than targeting DNA methylation alone[67]. As high doses of decitabine are cytotoxic[141], careful characterization of the desired therapeutic window will be important for future studies.

Elucidation of the role of L-2-HG in RCC epigenetic dysregulation has increased understanding of this disease process and identified potential therapeutic strategies. Interrogation of the epigenetic effects of L-2-HG demonstrated elevated levels of the trimethylated histone H3K27Me3, which corresponded with reduced levels of DNA 5hmC, suggesting L-2-HG-induced KDM inhibition in RCC[76]. Lowering L-2-HG levels in these cells leads to the re-expression of *H3K27Me3* target genes, as well as polycomb repressor complex 2 (*PRC2*) target genes, which encode a histone methyltransferase responsible for the repressive trimethylation of H327Me3 (REFS[76,227]). Inhibition of PRC2 via knockdown of the PRC2 catalytic subunit, *EZH2*, in RCC cells with high 2HG-levels, resulted in reduced H3K27Me3 levels, as well as reduced migratory abilities[76]. Furthermore, knockdown of *KDM6A* (also known as UTX), a known H3K27 demethylase, in *L2HGDH*-wild type RCC phenocopied the enhanced migratory properties of RCC cells with elevated L-2-HG levels, implicating *KDM6A* as a specific target for L-2-HG in RCC[76]. Mutations in *KDM6A* (predominantly somatic) have been identified in renal cancer[228,229], suggesting that chromatin remodelling via oncometabolites might recapitulate the effects of mutations in epigenetic modifiers in RCC. In other words, oncometabolites and chromatic modifiers may converge towards the same gene signature.

Owing to the identification of mutations in epigenetic regulators, such as KDM6A, in renal cancer[228,229], several studies have investigated the effects of DNMT inhibitors in this setting, with encouraging results. For example, DNMT inhibitors inhibited the growth of *VHL*-mutant and *VHL*-wild type RCC cell lines[230]. Dose-dependent re-expression of silenced genes was also observed with DNMT inhibition in several RCC cell lines[230,231]. DNMT inhibitors reversed the silencing methylation of interferon (IFN) response genes in RCC cells, enabling their re-expression and therefore augmenting IFN-induced apoptosis in vitro[232]. An early clinical study demonstrated the potential efficacy of combined DNMT inhibition and IFN therapy in patients with metastatic RCC[233]. Overall, these studies demonstrate that targeting epigenetic modifiers in RCC has antitumoural effects that may also potentiate and synergise with other adjunctive therapies such as IFN therapy. Given that oncometabolites and other mutated epigenetic modifiers in RCC may converge towards the same gene signature, these studies are especially relevant to therapeutic tactics for targeting aggressive oncometabolite-associated RCCs.

Use of oncometabolites as biomarkers

Oncometabolites and associated phenomena can also be exploited for their potential as biomarkers. Potential applications include the use of oncometabolite biomarkers in conjunction with clinical imaging and histology for diagnostic and prognostic assessments, as well as their application in surgery to optimize oncological resections.

Metabolic imaging

As oncometabolites accumulate in tissue to millimolar levels, their accumulation could be exploited to detect tumour masses using multiple metabolic imaging modalities. One advance has been the development of hyperpolarized magnetic resonance imaging (hpMRI)[234,235]. Use of hpMRI to image isotopically labelled ^{13}C-glutamine in an *IDH1*- and *IDH*2-mutant chondrosarcoma xenograft mouse model enabled the visualization of glutamine conversion to 2HG in real-time[209]. Most strikingly, hpMRI was able to capture the suppression of 2HG accumulation in response to IDH inhibition[209]. A similar study performed in a ccRCC xenograft model utilized labelled ^{13}C-pyruvate to visualize the metabolic response of the glycolytic flux to lactate in response to mTOR inhibitors[236]. Capitalizing on the unique oncometabolite properties in RCC, hpMRI has multiple potential applications. This technology can facilitate the diagnosis of oncometabolite-associated RCC subtypes such as L-2-HG-associated RCC, whilst concomitantly conferring prognostic information, for example, L-2-HG is associated with poor patient prognosis and tumour progression[76]. Dynamic assessment of oncometabolite levels in this setting could also be used to assess therapeutic efficacy, as well as to monitor progression or recurrence of the disease. In the wider context, utilizing hpMRI alongside selective tracers to identify malignant metabolic signatures would facilitate more robust diagnoses of small and/or indeterminate renal lesions. The largely non-invasive and safe, non-ionizing radioactive nature of hpMRI makes this imaging modality a very attractive tool for development and translation into clinical practice.

Other imaging modalities, such as proton magnetic resonance spectroscopy (1H-MRS) and positron emission tomography (PET), may also prove valuable in the diagnosis and management of oncometabolite-associated RCC. Successful detection of succinate using 1H-MRS in patients with a variety of SDH-deficient tumours[237,238], and of 2HG in IDH-mutant gliomas[239–241], has led to the use of 1H-MRS in clinical practice, including in disease monitoring of IDH-mutant gliomas[240]. Although the use of 1H-MRS to assess the metabolic profile has been explored in patients with RCC[242], the knowledge that the majority of oncometabolites have been implicated in RCC provides strong evidence for further investigation and may hold promise for patients with rare and aggressive RCC subtypes such as SDH-deficient tumours. In addition, a preclinical study capitalizing on glutamine reliance in several RCC subtypes demonstrated the ability to dynamically assess ccRCC metabolism in vivo using PET imaging with the radiotracer ^{18}F-(2 S,4 R)4-fluoroglutamine (18F-FGln)[210]. This approach could potentially be used to diagnose and stage RCCs, as well as to identify patients who are likely to respond to glutaminase inhibition[210].

Optimizing surgical margins

Oncometabolite-associated biomarkers may also prove indispensable for optimizing surgical oncology. For example, intraoperative mass spectrometry of 2HG has been used to guide brain tumour resections with promising results[243]. Detecting oncometabolites at tumour

resection margins or "molecular margins" identifies the presence of tumour cells and thus provides a straightforward guide for the need for further resections[243]. This technique provides information on metabolites within minutes and concurrently yields information about genotype, tumour classification and potentially also prognosis[243]. As multiple oncometabolites have been implicated in numerous RCC subtypes, including ccRCC[76], and partial nephrectomies are the gold-standard treatment for localized RCC[244], utilizing these methods may help to optimize surgical margins in patients with oncometabolite-associated RCC undergoing partial nephrectomies. Such optimization may prove to be of great benefit for patients, as positive surgical margins have been demonstrated to correlate with tumour recurrence[245]. Furthermore, given the highly aggressive phenotypes of FH-deficient and SDH-deficient RCCs and the fact that a substantial proportion of patients with these tumours present with bilateral disease, utilizing intraoperative mass spectrometry may assist in meticulous tumour resection to help preserve renal function.

Diagnostic and prognostic biomarkers. Unique oncometabolite properties such as post-translational modifications of proteins and metabolic rewiring can also be exploited for use as diagnostic or prognostic biomarkers. Capitalizing on the ability of fumarate to induce protein succination (2SC), detection of the distinct 2SC protein modification signature on immunohistochemistry or cyst staining has been corroborated to be a robust diagnostic biomarker of FH deficiency, for example, in patients with HLRCC, with ramifications for genetic testing[78,246]. In addition, metabolomic analyses of urine from FH1-deficient mice and growth media of FH-deficient cells revealed consistently elevated levels of argininosuccinate as a result of fumarate-induced reversal of ASL activity, raising its potential as a urinary biomarker for the early detection of FH-deficient renal cancer[102]. Although this biomarker awaits validation, the non-invasive nature of sampling, the specificity to FH-deficiency metabolism and the straightforward detection methods make this an exciting and attractive diagnostic biomarker.

Conclusions

Although in its infancy, the oncometabolite paradigm has been gathering momentum over the last decade, with a firm movement away from the traditional view of metabolism as a simple by-product of genetic perturbations that occur in cancer. A growing body of evidence has substantiated the roles of a small group of seemingly innocuous metabolites that, when aberrantly accumulated, are transformed into oncometabolites that can contribute to tumorigenesis and tumour progression. Given that RCC is an established metabolic disease process, the finding that multiple oncometabolites are implicated in renal cancer is unsurprising. In general, oncometabolites in RCC exert important effects on chromatin remodelling and epigenetic dysregulation leading to characteristic hypermethylated phenotypes, an EMT phenotypic switch and the propagation of a pseudohypoxic signature that contributes to the aggressive features of these RCC subtypes. Elucidating the roles of oncometabolites in oncogenesis will enable the exploitation of these molecules and their associated signalling pathways for multiple clinical applications, including the development of novel therapies and oncometabolite-associated biomarkers.

Published online: 21 October 2019

1. Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **68**, 394–424 (2018).
2. Bhatt, J. R. & Finelli, A. Landmarks in the diagnosis and treatment of renal cell carcinoma. *Nat. Rev. Urol.* **11**, 517–525 (2014).
3. Linehan, W. M., Walther, M. M. & Zbar, B. The genetic basis of cancer of the kidney. *J. Urol.* **170**, 2163–2172 (2003).
4. Moch, H., Cubilla, A. L., Humphrey, P. A., Reuter, V. E. & Ulbright, T. M. The 2016 WHO Classification of tumours of the urinary system and male genital organs — part a: renal, penile, and testicular tumours. *Eur. Urol.* **70**, 93–105 (2016).
5. Ricketts, C. J. et al. The cancer genome atlas comprehensive molecular characterization of renal cell carcinoma. *Cell Rep.* **0**, (2018).
6. Young, M. D. et al. Single-cell transcriptomes from human kidneys reveal the cellular identity of renal tumors. *Science* **361**, 594–599 (2018).
7. Mitchell, T. J. et al. Timing the landmark events in the evolution of clear cell renal cell cancer: TRACERx Renal. *Cell* **173**, 611–623 (2018).
8. Turajlic, S. et al. Deterministic evolutionary trajectories influence primary tumor growth: TRACERx renal. *Cell* **173**, 595–610 (2018).
9. Turajlic, S. et al. Tracking cancer evolution reveals constrained routes to metastases: TRACERx renal. *Cell* **173**, 581–594 (2018).
10. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature* **499**, 43–49 (2013).
11. Zbar, B., Brauch, H., Talmadge, C. & Linehan, M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. *Nature* **327**, 721 (1987).
12. Sato, Y. et al. Integrated molecular analysis of clear-cell renal cell carcinoma. *Nat. Genet.* **45**, 860–867 (2013).
13. Hosen, I. et al. TERT promoter mutations in clear cell renal cell carcinoma. *Int. J. Cancer* **136**, 2448–2452 (2015).
14. Shroff, E. H. et al. MYC oncogene overexpression drives renal cell carcinoma in a mouse model through glutamine metabolism. *Proc. Natl Acad. Sci. USA* **112**, 6539–6544 (2015).
15. Tang, S.-W. et al. MYC pathway is activated in clear cell renal cell carcinoma and essential for proliferation of clear cell renal cell carcinoma cells. *Cancer Lett.* **273**, 35–43 (2009).
16. The Multiple Leiomyoma Consortium. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat. Genet.* **30**, 406–410 (2002).
17. Linehan, W. M. & Ricketts, C. J. The metabolic basis of kidney cancer. *Semin. Cancer Biol.* **23**, 46–55 (2013).
18. Linehan, W. M., Srinivasan, R. & Schmidt, L. S. The genetic basis of kidney cancer: a metabolic disease. *Nat. Rev. Urol.* **7**, 277–285 (2010).
19. Jaakkola, P. et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science* **292**, 468–472 (2001).
20. Maxwell, P. H. et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399**, 271–275 (1999).
21. Pescador, N. et al. Hypoxia promotes glycogen accumulation through hypoxia inducible factor (HIF)-mediated induction of glycogen synthase 1. *PLOS ONE* **5**, e9644 (2010).
22. Semenza, G. L. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. *J. Clin. Invest.* **123**, 3664–3671 (2013).
23. Wise, D. R. et al. Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. *Proc. Natl. Acad. Sci. USA* **108**, 19611–19616 (2011).
24. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
25. Pavlova, N. N. & Thompson, C. B. The emerging hallmarks of cancer metabolism. *Cell Metab.* **23**, 27–47 (2016).
26. Isaacs, J. S. et al. HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. *Cancer Cell* **8**, 143–153 (2005).
27. Pollard, P. J. et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1α in tumours which result from germline FH and SDH mutations. *Hum. Mol. Genet.* **14**, 2231–2239 (2005).
28. Sudarshan, S. et al. Reduced expression of fumarate hydratase in clear cell renal cancer mediates HIF-2α accumulation and promotes migration and invasion. *PLOS ONE* **6**, e21037 (2011).
29. Selak, M. A. et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell* **7**, 77–85 (2005).
30. Brugarolas, J. B., Vazquez, F., Reddy, A., Sellers, W. R. & Kaelin, W. G. TSC2 regulates VEGF through mTOR-dependent and -independent pathways. *Cancer Cell* **4**, 147–158 (2003).
31. Li, B. et al. Fructose-1,6-bisphosphatase opposes renal carcinoma progression. *Nature* **513**, 251–255 (2014).
32. Du, W. et al. HIF drives lipid deposition and cancer in ccRCC via repression of fatty acid metabolism. *Nat. Commun.* **8**, 1–12 (2017).
33. Ward, P. S. & Thompson, C. B. Metabolic reprogramming: a cancer hallmark even Warburg

did not anticipate. *Cancer Cell* **21**, 297–308 (2012).
34. Goodacre, R., Vaidyanathan, S., Dunn, W. B., Harrigan, G. G. & Kell, D. B. Metabolomics by numbers: acquiring and understanding global metabolite data. *Trends Biotechnol.* **22**, 245–252 (2004).
35. Horgan, R. P. & Kenny, L. C. 'Omic' technologies: genomics, transcriptomics, proteomics and metabolomics. *Obstet. Gynaecol.* **13**, 189–195 (2011).
36. Gouirand, V., Guillaumond, F. & Vasseur, S. Influence of the tumor microenvironment on cancer cells metabolic reprogramming. *Front. Oncol* **8**, 117 (2018).
37. Yang, L. V. Tumor microenvironment and metabolism. *Int. J. Mol. Sci.* **18**, E2729 (2017).
38. Hakimi, A. A. et al. An integrated metabolic atlas of clear cell renal cell carcinoma. *Cancer Cell* **29**, 104–116 (2016).
39. Wettersten, H. I. et al. Grade-dependent metabolic reprogramming in kidney cancer revealed by combined proteomics and metabolomics analysis. *Cancer Res.* **75**, 2541–2552 (2015).
40. van der Mijn, J. C. et al. Novel drugs that target the metabolic reprogramming in renal cell cancer. *Cancer Metab.* **4**, 14 (2016).
41. Astuti, D. et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am. J. Hum. Genet.* **69**, 49–54 (2001).
42. Niemann, S. & Müller, U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat. Genet.* **26**, 268–270 (2000).
43. Warburg, O. The metabolism of carcinoma cells. *J. Cancer Res.* **9**, 148–163 (1925).
44. Warburg, O. On the origin of cancer cells. *Science* **123**, 309–314 (1956).
45. Racker, E. Bioenergetics and the problem of tumor growth. *Am. Sci.* **60**, 56–63 (1972).
46. Flier, J. S., Mueckler, M. M., Usher, P. & Lodish, H. F. Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. *Science* **235**, 1492–1495 (1987).
47. Frezza, C., Pollard, P. J. & Gottlieb, E. Inborn and acquired metabolic defects in cancer. *J. Mol. Med.* **89**, 213–220 (2011).
48. Haber, D. A. & Fearon, E. R. The promise of cancer genetics. *Lancet* **351**, SII1–SII8 (1998).
49. Liberti, M. V. & Locasale, J. W. The warburg effect: how does it benefit cancer cells? *Trends Biochem. Sci.* **41**, 211–218 (2016).
50. Baysal, B. E. et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* **287**, 848–851 (2000).
51. Cervera, A. M., Apostolova, N., Crespo, F. L., Mata, M. & McCreath, K. J. Cells silenced for SDHB expression display characteristic features of the tumor phenotype. *Cancer Res.* **68**, 4058–4067 (2008).
52. Dahia, P. L. M. et al. A HIF1α regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. *PLOS Genet.* **1**, (2005).
53. López-Jiménez, E. et al. Research resource: transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. *Mol. Endocrinol.* **24**, 2382–2391 (2010).
54. Pollard, P. et al. Evidence of increased microvessel density and activation of the hypoxia pathway in tumours from the hereditary leiomyomatosis and renal cell cancer syndrome. *J. Pathol.* **205**, 41–49 (2005).
55. Vanharanta, S. et al. Distinct expression profile in fumarate-hydratase-deficient uterine fibroids. *Hum. Mol. Genet.* **15**, 97–103 (2006).
56. Mohlin, S., Wigerup, C., Jögi, A. & Påhlman, S. Hypoxia, pseudohypoxia and cellular differentiation. *Exp. Cell Res.* **356**, 192–196 (2017).
57. Wigerup, C., Påhlman, S. & Bexell, D. Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. *Pharmacol. Ther.* **164**, 152–169 (2016).
58. Dang, L. et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **462**, 739–744 (2009).
59. Achouri, Y. et al. Identification of a dehydrogenase acting on D-2-hydroxyglutarate. *Biochem. J.* **381**, 35–42 (2004).
60. Collins, R. R. J., Patel, K., Putnam, W. C., Kapur, P. & Rakheja, D. Oncometabolites: a new paradigm for oncology, metabolism, and the clinical laboratory. *Clin. Chem.* **63**, 1812–1820 (2017).
61. Linster, C. L., Van Schaftingen, E. & Hanson, A. D. Metabolite damage and its repair or pre-emption. *Nat. Chem. Biol.* **9**, 72–80 (2013).
62. Struys, E. A. 2-Hydroxyglutarate is not a metabolite; D-2-hydroxyglutarate and L-2-hydroxyglutarate are! *Proc. Natl Acad. Sci. USA* **110**, E4939 (2013).
63. Aghili, M., Zahedi, F. & Rafiee, E. Hydroxyglutaric aciduria and malignant brain tumor: a case report and literature review. *J. Neurooncol.* **91**, 233–236 (2009).
64. Moroni, I. et al. L-2-hydroxyglutaric aciduria and brain malignant tumors: a predisposing condition? *Neurology* **62**, 1882–1884 (2004).
65. Rogers, R. E. et al. Wilms tumor in a child with L-2-hydroxyglutaric aciduria. *Pediatr. Dev. Pathol.* **13**, 408–411 (2010).
66. Kranendijk, M. et al. IDH2 Mutations in patients with D-2-hydroxyglutaric aciduria. *Science* **330**, 336–336 (2010).
67. Morin, A., Letouzé, E., Gimenez-Roqueplo, A.-P. & Favier, J. Oncometabolites-driven tumorigenesis: from genetics to targeted therapy. *Int. J. Cancer* **135**, 2237–2248 (2014).
68. Sciacovelli, M. & Frezza, C. Oncometabolites: unconventional triggers of oncogenic signalling cascades. *Free Radic. Biol. Med.* **100**, 175–181 (2016).
69. Castro-Vega, L. J. et al. Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. *Hum. Mol. Genet.* **23**, 2440–2446 (2014).
70. Kaelin, W. G. SDH5 mutations and familial paraganglioma: somewhere Warburg is smiling. *Cancer Cell* **16**, 180–182 (2009).
71. Xu, W. et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell* **19**, 17–30 (2011).
72. Yan, H. et al. IDH1 and IDH2 mutations in gliomas. *N. Engl. J. Med.* **360**, 765–773 (2009).
73. Gross, S. et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *J. Exp. Med.* **207**, 339–344 (2010).
74. Ward, P. S. et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* **17**, 225–234 (2010).
75. Mukherjee, P. K. et al. Metabolomic analysis identifies differentially produced oral metabolites, including the oncometabolite 2-hydroxyglutarate, in patients with head and neck squamous cell carcinoma. *BBA Clin.* **7**, 8–15 (2017).
76. Shelar, S. et al. Biochemical and epigenetic insights into l-2-hydroxyglutarate, a potential therapeutic target in renal cancer. *Clin. Cancer Res.* **24**, 6433–6446 (2018).
77. Sciacovelli, M. et al. Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition. *Nature* **537**, 544–547 (2016).
78. Bardella Chiara et al. Aberrant succination of proteins in fumarate hydratase-deficient mice and HLRCC patients is a robust biomarker of mutation status. *J. Pathol.* **225**, 4–11 (2011).
79. Kinch, L., Grishin, N. V. & Brugarolas, J. Succination of Keap1 and activation of Nrf2-dependent antioxidant pathways in FH-deficient papillary renal cell carcinoma type-2. *Cancer Cell* **20**, 418–420 (2011).
80. Gottlieb, E. & Tomlinson, I. P. M. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat. Rev. Cancer* **5**, 857–866 (2005).
81. Knudson, A. G. Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl Acad. Sci. USA* **68**, 820–823 (1971).
82. Vranken, J. G. V., Na, U., Winge, D. R. & Rutter, J. Protein-mediated assembly of succinate dehydrogenase and its cofactors. *Crit. Rev. Biochem. Mol. Biol.* **50**, 168–180 (2015).
83. Malinoc, A. et al. Biallelic inactivation of the SDHC gene in renal carcinoma associated with paraganglioma syndrome type 3. *Endocr. Relat. Cancer* **19**, 283–290 (2012).
84. Ni, Y. et al. Germline mutations and variants in the succinate dehydrogenase genes in Cowden and Cowden-like syndromes. *Am. J. Hum. Genet.* **83**, 261–268 (2008).
85. Lee, C.-H. et al. Persistent severe hyperlactatemia and metabolic derangement in lethal *SDHB*-mutated metastatic kidney cancer: clinical challenges and examples of extreme Warburg effect. *JCO Precis. Oncol.* **1**, 1–14 (2017).
86. McEvoy, C. R. et al. SDH-deficient renal cell carcinoma associated with biallelic mutation in succinate dehydrogenase A: comprehensive genetic profiling and its relation to therapy response. *NPJ Precis. Oncol.* **2**, 9 (2018).
87. Vanharanta, S. et al. Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am. J. Hum. Genet.* **74**, 153–159 (2004).
88. Gill, A. J. et al. Succinate dehydrogenase (SDH)-deficient renal carcinoma: a morphologically distinct entity. *Am. J. Surg. Pathol.* **38**, 1588–1602 (2014).
89. Ricketts, C. J. et al. Succinate dehydrogenase kidney cancer: an aggressive example of the Warburg effect in cancer. *J. Urol.* **188**, 2063–2071 (2012).
90. Williamson, S. R. et al. Succinate dehydrogenase-deficient renal cell carcinoma: detailed characterization of 11 tumors defining a unique subtype of renal cell carcinoma. *Mod. Pathol.* **28**, 80–94 (2015).
91. Alam, N. A. et al. Genetic and functional analyses of FH mutations in multiple cutaneous and uterine leiomyomatosis, hereditary leiomyomatosis and renal cancer, and fumarate hydratase deficiency. *Hum. Mol. Genet.* **12**, 1241–1252 (2003).
92. Lehtonen, H. J. et al. Increased risk of cancer in patients with fumarate hydratase germline mutation. *J. Med. Genet.* **43**, 523–526 (2006).
93. Linehan, W. M. & Rouault, T. A. Molecular pathways: fumarate hydratase-deficient kidney cancer — targeting the Warburg effect in cancer. *Clin. Cancer Res.* **19**, 3345–3352 (2013).
94. Schmidt, L. S. & Linehan, W. M. Hereditary leiomyomatosis and renal cell carcinoma. *Int. J. Nephrol. Renovasc. Dis.* **7**, 253–260 (2014).
95. Schmidt, L. S. & Linehan, W. M. Genetic predisposition to kidney cancer. *Semin. Oncol.* **43**, 566–574 (2016).
96. Clark, G. R. et al. Germline FH mutations presenting with pheochromocytoma. *J. Clin. Endocrinol. Metab.* **99**, E2046–E2050 (2014).
97. Neumann, H. P. H. et al. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *JAMA* **292**, 943–951 (2004).
98. Allegri, G. et al. Fumaric aciduria: an overview and the first Brazilian case report. *J. Inherit. Metab. Dis.* **33**, 411–419 (2010).
99. Kerrigan, J. F., Aleck, K. A., Tarby, T. J., Bird, C. R. & Heidenreich, R. A. Fumaric aciduria: clinical and imaging features. *Ann. Neurol.* **47**, 583–588 (2000).
100. Loeffen, J., Smeets, R., Voit, T., Hoffmann, G. & Smeitink, J. Fumarase deficiency presenting with periventricular cysts. *J. Inherit. Metab. Dis.* **28**, 799–800 (2005).
101. Haigis, K. M., Cichowski, K. & Elledge, S. J. Tissue-specificity in cancer: the rule, not the exception. *Science* **363**, 1150–1151 (2019).
102. Zheng, L. et al. Reversed argininosuccinate lyase activity in fumarate hydratase-deficient cancer cells. *Cancer Metab.* **1**, 12 (2013).
103. Wallace, D. C. Mitochondria and cancer. *Nat. Rev. Cancer* **12**, 685–698 (2012).
104. Parsons, D. W. et al. An integrated genomic analysis of human glioblastoma multiforme. *Science* **321**, 1807–1812 (2008).
105. Marcucci, G. et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *J. Clin. Oncol.* **28**, 2348–2355 (2010).
106. Lin, A.-P. et al. D2HGDH regulates alpha-ketoglutarate levels and dioxygenase function by modulating IDH2. *Nat. Commun.* **6**, 7768 (2015).
107. Fan, J. et al. Human phosphoglycerate dehydrogenase produces the oncometabolite d-2-hydroxyglutarate. *ACS Chem. Biol.* **10**, 510–516 (2015).
108. Gao, J. et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **6**, pl1 (2013).
109. Lee, J. H. et al. IDH1 R132C mutation is detected in clear cell hepatocellular carcinoma by pyrosequencing. *World J. Surg. Oncol.* **15**, 82 (2017).
110. Shim, E.-H. et al. l-2-Hydroxyglutarate: an epigenetic modifier and putative oncometabolite in renal cancer. *Cancer Discov.* **4**, 1290–1298 (2014).
111. Mitchell, T. J., Rossi, S. H., Klatte, T. & Stewart, G. D. Genomics and clinical correlates of renal cell carcinoma. *World J. Urol.* **36**, 1899–1911 (2018).
112. Burr, S. P. et al. Mitochondrial protein lipoylation and the 2-oxoglutarate dehydrogenase complex controls

HIF1α stability in aerobic conditions. *Cell Metab.* **24**, 740–752 (2016).
113. Ni, M. et al. Functional assessment of lipoyltransferase-1 deficiency in cells, mice, and humans. *Cell Rep.* **27**, 1376–1386.e6 (2019).
114. Baker, P. R. et al. Variant non ketotic hyperglycinemia is caused by mutations in LIAS, BOLA3 and the novel gene GLRX5. *Brain* **137**, 366–379 (2014).
115. Losman, J.-A. et al. (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science* **339**, 1621–1625 (2013).
116. Intlekofer, A. M. et al. Hypoxia induces production of l-2-hydroxyglutarate. *Cell Metab.* **22**, 304–311 (2015).
117. Oldham, W. M., Clish, C. B., Yang, Y. & Loscalzo, J. Hypoxia-mediated increases in l-2-hydroxyglutarate coordinate the metabolic response to reductive stress. *Cell Metab.* **22**, 291–303 (2015).
118. Nadtochiy, S. M. et al. Metabolomic profiling of the heart during acute ischemic preconditioning reveals a role for SIRT1 in rapid cardioprotective metabolic adaptation. *J. Mol. Cell Cardiol.* **88**, 64–72 (2015).
119. Nadtochiy, S. M. et al. Acidic pH Is a metabolic switch for 2-hydroxyglutarate generation and signaling. *J. Biol. Chem.* **291**, 20188–20197 (2016).
120. Rodenhizer, D. et al. A 3D engineered tumour for spatial snap-shot analysis of cell metabolism and phenotype in hypoxic gradients. *Nat. Mater.* **15**, 227–234 (2016).
121. Chouchani, E. T. et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature* **515**, 431–435 (2014).
122. Sapieha, P. et al. The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. *Nat. Med.* **14**, 1067–1076 (2008).
123. Zhang, J. et al. Accumulation of succinate in cardiac ischemia primarily occurs via canonical krebs cycle activity. *Cell Rep.* **23**, 2617–2628 (2018).
124. Kohlhauer Matthias *et al*. Metabolomic profiling in acute ST-segment–elevation myocardial infarction identifies succinate as an early marker of human ischemia–reperfusion injury. *J. Am. Heart Assoc*. **7**, e007546
125. O'Flaherty, L. et al. Dysregulation of hypoxia pathways in fumarate hydratase-deficient cells is independent of defective mitochondrial metabolism. *Hum. Mol. Genet.* **19**, 3844–3851 (2010).
126. Selak, M. A., Durán, R. V. & Gottlieb, E. Redox stress is not essential for the pseudo-hypoxic phenotype of succinate dehydrogenase deficient cells. *Biochim. Biophys. Acta* **1757**, 567–572 (2006).
127. Puisségur, M.-P. et al. miR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity. *Cell Death Differ.* **18**, 465–478 (2011).
128. Blatnik, M., Frizzell, N., Thorpe, S. R. & Baynes, J. W. Inactivation of glyceraldehyde-3-phosphate dehydrogenase by fumarate in diabetes: formation of s-(2-succinyl)cysteine, a novel chemical modification of protein and possible biomarker of mitochondrial stress. *Diabetes* **57**, 41–49 (2008).
129. Frizzell, N., Thomas, S. A., Carson, J. A. & Baynes, J. W. Mitochondrial stress causes increased succination of proteins in adipocytes in response to glucotoxicity. *Biochem. J.* **445**, 247–254 (2012).
130. Gao, C.-L. et al. Mitochondrial dysfunction is induced by high levels of glucose and free fatty acids in 3T3-L1 adipocytes. *Mol. Cell. Endocrinol.* **320**, 25–33 (2010).
131. Yang, M. et al. The succinated proteome of FH-mutant tumours. *Metabolites* **4**, 640–654 (2014).
132. Thomas, S. A., Storey, K. B., Baynes, J. W. & Frizzell, N. Tissue distribution of S-(2-succino)cysteine (2SC), a biomarker of mitochondrial stress in obesity and diabetes. *Obesity* **20**, 263–269 (2012).
133. Guo, Y. et al. Succinate and its G-protein-coupled receptor stimulates osteoclastogenesis. *Nat. Commun.* **8**, 15621 (2017).
134. Mitchell, T. & Darley-Usmar, V. Metabolic syndrome and mitochondrial dysfunction: insights from pre-clinical studies with a mitochondrially targeted antioxidant. *Free. Radic. Biol. Med.* **52**, 838–840 (2012).
135. Zhang, G.-M., Zhu, Y. & Ye, D.-W. Metabolic syndrome and renal cell carcinoma. *World J. Surg. Oncol.* **12**, 236 (2014).
136. Häggström, C. et al. Metabolic factors associated with risk of renal cell carcinoma. *PLOS ONE* **8**, e57475 (2013).
137. Mills, E. L. et al. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell* **167**, 457–470.e13 (2016).
138. Tannahill, G. et al. Succinate is a danger signal that induces IL-1β via HIF-1α. *Nature* **496**, 238–242 (2013).
139. Kaelin, W. G. Cancer and altered metabolism: potential importance of hypoxia-inducible factor and 2-oxoglutarate-dependent dioxygenases. *Cold Spring Harb. Symp. Quant. Biol.* **76**, 335–345 (2011).
140. Xiao, M. et al. Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. *Genes Dev.* **26**, 1326–1338 (2012).
141. Letouzé, E. et al. SDH mutations establish a hypermethylator phenotype in paraganglioma. *Cancer Cell* **23**, 739–752 (2013).
142. Lu, C. et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature* **483**, 474–478 (2012).
143. Laukka, T. et al. Fumarate and succinate regulate expression of hypoxia-inducible genes via TET enzymes. *J. Biol. Chem.* **291**, 4256–4265 (2016).
144. Guzy, R. D., Sharma, B., Bell, E., Chandel, N. S. & Schumacker, P. T. Loss of the SdhB, but not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Mol. Cell Biol.* **28**, 718–731 (2008).
145. Adam, J. et al. Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signaling. *Cancer Cell* **20**, 524–537 (2011).
146. Chowdhury, R. et al. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep.* **12**, 463–469 (2011).
147. Koivunen, P. et al. Transformation by the (*R*)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. *Nature* **483**, 484–488 (2012).
148. Tarhonskaya, H. et al. Non-enzymatic chemistry enables 2-hydroxyglutarate-mediated activation of 2-oxoglutarate oxygenases. *Nat. Commun.* **5**, 3423 (2014).
149. Cedar, H. & Bergman, Y. Linking DNA methylation and histone modification: patterns and paradigms. *Nat. Rev. Genet.* **10**, 295–304 (2009).
150. Feinberg, A. P., Ohlsson, R. & Henikoff, S. The epigenetic progenitor origin of human cancer. *Nat. Rev. Genet.* **7**, 21–33 (2006).
151. Jones, P. A. & Baylin, S. B. The epigenomics of cancer. *Cell* **128**, 683–692 (2007).
152. Figueroa, M. E. et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* **18**, 553–567 (2010).
153. Lu, C. et al. Induction of sarcomas by mutant IDH2. *Genes Dev.* **27**, 1986–1998 (2013).
154. Turcan, S. et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* **483**, 479–483 (2012).
155. Aspuria, P.-J. P. et al. Succinate dehydrogenase inhibition leads to epithelial-mesenchymal transition and reprogrammed carbon metabolism. *Cancer Metab.* **2**, 21 (2014).
156. Enane, F. O., Saunthararajah, Y. & Korc, M. Differentiation therapy and the mechanisms that terminate cancer cell proliferation without harming normal cells. *Cell Death Dis.* **9**, 1–15 (2018).
157. Zhou, D., Luo, Y., Dingli, D. & Traulsen, A. The invasion of de-differentiating cancer cells into hierarchical tissues. *PLOS Computational Biol.* **15**, e1007167 (2019).
158. Ahuja, N. et al. Association between CpG island methylation and microsatellite instability in colorectal cancer. *Cancer Res.* **57**, 3370–3374 (1997).
159. Issa, J.-P. CpG island methylator phenotype in cancer. *Nat. Rev. Cancer* **4**, 988–993 (2004).
160. Noushmehr, H. et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* **17**, 510–522 (2010).
161. Linehan, W. M. et al. Comprehensive molecular characterization of papillary renal cell carcinoma. *N. Engl. J. Med.* **374**, 135–145 (2016).
162. Chan, M. M. Y. et al. Cascade fumarate hydratase mutation screening allows early detection of kidney tumour: a case report. *BMC Med. Genet.* **18**, 79 (2017).
163. Ooi, A. et al. An antioxidant response phenotype shared between hereditary and sporadic type 2 papillary renal cell carcinoma. *Cancer Cell* **20**, 511–523 (2011).
164. Sourbier, C. et al. Targeting ABL1-mediated oxidative stress adaptation in fumarate hydratase-deficient cancer. *Cancer Cell* **26**, 840–850 (2014).
165. Shanmugasundaram, K. et al. The oncometabolite fumarate promotes pseudohypoxia through noncanonical activation of NF-κB signaling. *J. Biol. Chem.* **289**, 24691–24699 (2014).
166. van Uden, P., Kenneth, N. S. & Rocha, S. Regulation of hypoxia-inducible factor-1alpha by NF-kappaB. *Biochem. J.* **412**, 477–484 (2008).
167. Tong, W.-H. et al. The glycolytic shift in fumarate-hydratase-deficient kidney cancer lowers AMPK levels, increases anabolic propensities and lowers cellular iron levels. *Cancer Cell* **20**, 315–327 (2011).
168. Bratslavsky, G., Sudarshan, S., Neckers, L. & Linehan, W. M. Pseudohypoxic pathways in renal cell carcinoma. *Clin. Cancer Res.* **13**, 4667–4671 (2007).
169. Sullivan, L. B. et al. The proto-oncometabolite fumarate binds glutathione to amplify ROS-dependent signaling. *Mol. Cell* **51**, 236–248 (2013).
170. Zheng, L. et al. Fumarate induces redox-dependent senescence by modifying glutathione metabolism. *Nat. Commun.* **6**, 6001 (2015).
171. Campisi, J. Aging, cellular senescence, and cancer. *Annu. Rev. Physiol.* **75**, 685–705 (2013).
172. van Deursen, J. M. The role of senescent cells in ageing. *Nature* **509**, 439–446 (2014).
173. Fourquet, S., Guerois, R., Biard, D. & Toledano, M. B. Activation of NRF2 by nitrosative agents and H_2O_2 involves KEAP1 disulfide formation. *J. Biol. Chem.* **285**, 8463–8471 (2010).
174. Jin, L. et al. Glutamate dehydrogenase 1 signals through antioxidant glutathione peroxidase 1 to regulate redox homeostasis and tumor growth. *Cancer Cell* **27**, 257–270 (2015).
175. Hensley, C. T., Wasti, A. T. & DeBerardinis, R. J. Glutamine and cancer: cell biology, physiology, and clinical opportunities. *J. Clin. Invest.* **123**, 3678–3684 (2013).
176. Mullen, A. R. et al. Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature* **481**, 385–388 (2012).
177. Xu, Y. et al. Pathologic oxidation of ptpn12 underlies abl1 phosphorylation in hereditary leiomyomatosis and renal cell carcinoma. *Cancer Res.* **78**, 6539–6548 (2018).
178. Shambaugh, G. E. Urea biosynthesis I. The urea cycle and relationships to the citric acid cycle. *Am. J. Clin. Nutr.* **30**, 2083–2087 (1977).
179. Frezza, C. et al. Haem oxygenase is synthetically lethal with the tumour suppressor fumarate hydratase. *Nature* **477**, 225–228 (2011).
180. Mu, X. et al. Oncometabolite succinate promotes angiogenesis by upregulating VEGF expression through GPR91-mediated STAT3 and ERK activation. *Oncotarget* **8**, 13174–13185 (2017).
181. Baumbach, L., Leyssac, P. P. & Skinner, S. L. Studies on renin release from isolated superfused glomeruli: effects of temperature, urea, ouabain and ethacrynic acid. *J. Physiol.* **258**, 243–256 (1976).
182. He, W. et al. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* **429**, 188–193 (2004).
183. Toma, I. et al. Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney. *J. Clin. Invest.* **118**, 2526–2534 (2008).
184. Aguiar, C. J. et al. Succinate causes pathological cardiomyocyte hypertrophy through GPR91 activation. *Cell Commun. Signal.* **12**, 78 (2014).
185. Correa, P. R. A. V. et al. Succinate is a paracrine signal for liver damage. *J. Hepatol.* **47**, 262–269 (2007).
186. Jiang, S. & Yan, W. Succinate in the cancer-immune cycle. *Cancer Lett.* **390**, 45–47 (2017).
187. Mills, E. & O'Neill, L. A. J. Succinate: a metabolic signal in inflammation. *Trends Cell Biol.* **24**, 313–320 (2014).
188. Zhang, Z. et al. Identification of lysine succinylation as a new post-translational modification. *Nat. Chem. Biol.* **7**, 58–63 (2011).
189. Li, F. et al. NADP+-IDH mutations promote hypersuccinylation that impairs mitochondria respiration and induces apoptosis resistance. *Mol. Cell* **60**, 661–675 (2015).
190. Xie, Z. et al. Lysine succinylation and lysine malonylation in histones. *Mol. Cell Proteom.* **11**, 100–107 (2012).

191. Park, J. et al. SIRT5-mediated lysine desuccinylation impacts diverse metabolic pathways. *Mol. Cell* **50**, 919–930 (2013).
192. Frezza, C. Mitochondrial metabolites: undercover signalling molecules. *Interface Focus* **7**, 20160100 (2017).
193. Cardaci, S. et al. Pyruvate carboxylation enables growth of SDH-deficient cells by supporting aspartate biosynthesis. *Nat. Cell Biol.* **17**, 1317–1326 (2015).
194. Lussey-Lepoutre, C. et al. Loss of succinate dehydrogenase activity results in dependency on pyruvate carboxylation for cellular anabolism. *Nat. Commun.* **6**, 8784 (2015).
195. Su, R. et al. R-2HG exhibits anti-tumor activity by targeting FTO/m6A/MYC/CEBPA Signaling. *Cell* **172**, 90–105.e23 (2018).
196. Jia, G. et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat. Chem. Biol.* **7**, 885–887 (2011).
197. Li, Z. et al. FTO plays an oncogenic role in acute myeloid leukemia as a n6-methyladenosine rna demethylase. *Cancer Cell* **31**, 127–141 (2017).
198. Fu, X. et al. 2-Hydroxyglutarate inhibits ATP synthase and mTOR signaling. *Cell Metab.* **22**, 508–515 (2015).
199. Cancer Genome Atlas Research Network. et al. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N. Engl. J. Med.* **372**, 2481–2498 (2015).
200. Colvin, H. et al. Oncometabolite D-2-hydroxyglurate directly induces epithelial-mesenchymal transition and is associated with distant metastasis in colorectal cancer. *Sci. Rep.* **6**, 36289 (2016).
201. Adam, J. et al. A role for cytosolic fumarate hydratase in urea cycle metabolism and renal neoplasia. *Cell Rep.* **3**, 1440–1448 (2013).
202. Nassereddine, S., Lap, C. J., Haroun, F. & Tabbara, I. The role of mutant IDH1 and IDH2 inhibitors in the treatment of acute myeloid leukemia. *Ann. Hematol.* **96**, 1983–1991 (2017).
203. Kats, L. M. et al. A pharmacogenomic approach validates AG-221 as an effective and on-target therapy in IDH2 mutant AML. *Leukemia* **31**, 1466–1470 (2017).
204. Kernytsky, A. et al. IDH2 mutation-induced histone and DNA hypermethylation is progressively reversed by small-molecule inhibition. *Blood* **125**, 296–303 (2015).
205. Wang, F. et al. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science* **340**, 622–626 (2013).
206. Birendra, K. C. & DiNardo, C. D. Evidence for clinical differentiation and differentiation syndrome in patients with acute myeloid leukemia and IDH1 mutations treated with the targeted mutant IDH1 inhibitor, AG-120. *Clin. Lymphoma. Myeloma. Leuk.* **16**, 460–465 (2016).
207. Norsworthy, K. J. et al. FDA approval summary: ivosidenib for relapsed or refractory acute myeloid leukemia with an isocitrate dehydrogenase-1 mutation. *Clin. Cancer Res.* **25**, 3205–3209 (2019).
208. Terunuma, A. et al. MYC-driven accumulation of 2-hydroxyglutarate is associated with breast cancer prognosis. *J. Clin. Invest.* **124**, 398–412 (2014).
209. Salamanca-Cardona, L. et al. In vivo imaging of glutamine metabolism to the oncometabolite 2-hydroxyglutarate in idh1/2 mutant tumors. *Cell Metab.* **26**, 830–841.e3 (2017).
210. Abu Aboud, O. et al. Glutamine addiction in kidney cancer suppresses oxidative stress and can be exploited for real-time imaging. *Cancer Res.* **77**, 6746–6758 (2017).
211. Gameiro, P. A. et al. In vivo HIF-mediated reductive carboxylation is regulated by citrate levels and sensitizes VHL-deficient cells to glutamine deprivation. *Cell Metab* **17**, 372–385 (2013).
212. Raczka, A. M. & Reynolds, P. A. Glutaminase inhibition in renal cell carcinoma therapy. *Cancer Drug Resist.* **2**, 356–364 (2019).
213. Song, M., Kim, S.-H., Im, C. Y. & Hwang, H.-J. Recent development of small molecule glutaminase inhibitors. *Curr. Top Med. Chem.* **18**, 432–443 (2018).
214. Tannir, N. M. et al. CANTATA: a randomized phase 2 study of CB-839 in combination with cabozantinib vs. placebo with cabozantinib in patients with advanced/metastatic renal cell carcinoma. *J. Clin. Oncol.* **36**, TPS4601–TPS4601 (2018).
215. Vancura, A., Bu, P., Bhagwat, M., Zeng, J. & Vancurova, I. Metformin as an anticancer agent. *Trends Pharmacol. Sci.* **39**, 867–878 (2018).
216. Brière, J.-J. et al. Mitochondrial succinate is instrumental for HIF1alpha nuclear translocation in SDHA-mutant fibroblasts under normoxic conditions. *Hum. Mol. Genet.* **14**, 3263–3269 (2005).
217. MacKenzie, E. D. et al. Cell-permeating alpha-ketoglutarate derivatives alleviate pseudohypoxia in succinate dehydrogenase-deficient cells. *Mol. Cell. Biol.* **27**, 3282–3289 (2007).
218. Matsumoto, K. et al. 2-Oxoglutarate downregulates expression of vascular endothelial growth factor and erythropoietin through decreasing hypoxia-inducible factor-1alpha and inhibits angiogenesis. *J. Cell. Physiol.* **209**, 333–340 (2006).
219. Matsumoto, K. et al. Antitumor effects of 2-oxoglutarate through inhibition of angiogenesis in a murine tumor model. *Cancer Science* **100**, 1639–1647 (2009).
220. Tennant, D. A. et al. Reactivating HIF prolyl hydroxylases under hypoxia results in metabolic catastrophe and cell death. *Oncogene* **28**, 4009–4021 (2009).
221. Chen, W. et al. Targeting renal cell carcinoma with a HIF-2 antagonist. *Nature* **539**, 112–117 (2016).
222. Cho, H. et al. On-target efficacy of a HIF-2α antagonist in preclinical kidney cancer models. *Nature* **539**, 107–111 (2016).
223. Courtney, K. D. et al. Phase I dose-escalation trial of pt2385, a first-in-class hypoxia-inducible factor-2α antagonist in patients with previously treated advanced clear cell renal cell carcinoma. *J. Clin. Oncol.* **36**, 867–874 (2018).
224. Fenaux, P. et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol.* **10**, 223–232 (2009).
225. Gurion, R. et al. 5-azacitidine prolongs overall survival in patients with myelodysplastic syndrome — a systematic review and meta-analysis. *Haematologica* **95**, 303–310 (2010).
226. Turcan, S. et al. Efficient induction of differentiation and growth inhibition in IDH1 mutant glioma cells by the DNMT inhibitor decitabine. *Oncotarget* **4**, 1729–1736 (2013).
227. Yoo, K. H. & Hennighausen, L. EZH2 methyltransferase and H3K27 methylation in breast cancer. *Int. J. Biol. Sci.* **8**, 59–65 (2012).
228. Dalgliesh, G. L. et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature* **463**, 360–363 (2010).
229. van Haaften, G. et al. Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. *Nat. Genet.* **41**, 521–523 (2009).
230. Hu, C. Y. et al. Kidney cancer is characterized by aberrant methylation of tissue-specific enhancers that are prognostic for overall survival. *Clin. Cancer Res.* **20**, 4349–4360 (2014).
231. To, K. K. W., Zhan, Z. & Bates, S. E. Aberrant promoter methylation of the ABCG2 gene in renal carcinoma. *Mol. Cell Biol.* **26**, 8572–8585 (2006).
232. Reu, F. J. et al. Overcoming resistance to interferon-induced apoptosis of renal carcinoma and melanoma cells by DNA demethylation. *J. Clin. Oncol.* **24**, 3771–3779 (2006).
233. Amato, R. J. et al. MG98, a second-generation DNMT1 inhibitor, in the treatment of advanced renal cell carcinoma. *Cancer Invest.* **30**, 415–421 (2012).
234. Brindle, K. M. Imaging metabolism with hyperpolarized (13)C-labeled cell substrates. *J. Am. Chem. Soc.* **137**, 6418–6427 (2015).
235. Skinner, J. G. et al. Metabolic and molecular imaging with hyperpolarised tracers. *Mol. Imaging Biol.* **20**, 902–918 (2018).
236. Dong, Y. et al. Hyperpolarized MRI visualizes Warburg effects and predicts treatment response to mTOR inhibitors in patient-derived ccRCC xenograft models. *Cancer Res.* **79**, 242–250 (2018).
237. Casey, R. T. et al. Translating in vivo metabolomic analysis of succinate dehydrogenase deficient tumours into clinical utility. *JCO Precis. Oncol.* **2**, 1–12 (2018).
238. Lussey-Lepoutre, C. et al. In vivo detection of succinate by magnetic resonance spectroscopy as a hallmark of sdhx mutations in paraganglioma. *Clin. Cancer Res.* **22**, 1120–1129 (2016).
239. Bisdas, S. et al. MR spectroscopy for in vivo assessment of the oncometabolite 2-hydroxyglutarate and its effects on cellular metabolism in human brain gliomas at 9.4T. *J. Magn. Reson. Imaging* **44**, 823–833 (2016).
240. de la Fuente, M. I. et al. Integration of 2-hydroxyglutarate-proton magnetic resonance spectroscopy into clinical practice for disease monitoring in isocitrate dehydrogenase-mutant glioma. *Neuro Oncol.* **18**, 283–290 (2016).
241. Kim, I.-Y., Suh, S.-H., Lee, I.-K. & Wolfe, R. R. Applications of stable, nonradioactive isotope tracers in in vivo human metabolic research. *Exp. Mol. Med.* **48**, e203 (2016).
242. Süllentrop, F., Hahn, J. & Moka, D. In vitro and in vivo 1h-mr spectroscopic examination of the renal cell carcinoma. *Int. J. Biomed. Sci.* **8**, 94–108 (2012).
243. Santagata, S. et al. Intraoperative mass spectrometry mapping of an onco-metabolite to guide brain tumor surgery. *Proc. Natl Acad. Sci. USA* **111**, 11121–11126 (2014).
244. Ljungberg, B. et al. EAU guidelines on renal cell carcinoma: 2014 update. *Eur. Urol.* **67**, 913–924 (2015).
245. Petros, F. G. et al. Oncologic outcomes of patients with positive surgical margin after partial nephrectomy: a 25-year single institution experience. *World J. Urol.* **36**, 1093–1101 (2018).
246. Maxwell, P. H. Seeing the smoking gun: a sensitive and specific method to visualize loss of the tumour suppressor, fumarate hydratase, in human tissues. *J. Pathol.* **225**, 1–3 (2011).
247. Yang, M., Soga, T., Pollard, P. J. & Adam, J. The emerging role of fumarate as an oncometabolite. *Front. Oncol.* **2**, 85 (2012).
248. Gupta, S. et al. Primary renal paragangliomas and renal neoplasia associated with pheochromocytoma/paraganglioma: analysis of von Hippel-Lindau (VHL), succinate dehydrogenase (SDHX) and transmembrane protein 127 (TMEM127). *Endocr. Pathol.* **28**, 253–268 (2017).
249. Ozluk, Y. et al. Renal carcinoma associated with a novel succinate dehydrogenase A mutation: a case report and review of literature of a rare subtype of renal carcinoma. *Hum. Pathol.* **46**, 1951–1955 (2015).
250. Ricketts, C. J. et al. Tumor risks and genotype–phenotype–proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. *Hum. Mutat.* **31**, 41–51 (2010).
251. Sanchez, D. J. & Simon, M. C. Genetic and metabolic hallmarks of clear cell renal cell carcinoma. *Biochim. Biophys. Acta* **1870**, 23–31 (2018).
252. Gudbjartsson, T. et al. Histological subtyping and nuclear grading of renal cell carcinoma and their implications for survival: a retrospective nation-wide study of 629 patients. *Eur. Urol.* **48**, 593–600 (2005).

Acknowledgements
C.Y. is funded by the Wellcome Trust and The Urology Foundation. C.F. is supported by the Medical Research Council, grant MRC_MC_UU_12022/6.

Author contributions
C.Y. C.F. and G.D.S. researched the data for the article and made substantial contributions to discussions of the content. C.Y. wrote the manuscript. C.Y. G.D.S. and C.F. reviewed and edited the manuscript before submission.

Competing interests
C.Y. declares no competing interests. G.D.S. has received educational grants from Pfizer, AstraZeneca and Intuitive Surgical, consultancy fees from Merck, Pfizer, EUSA Pharma and CMR Surgical, travel expenses from Pfizer and speaker fees from Pfizer. C.F. is an adviser of Istesso Limited and a member of the Scientific Advisory Board of Owlstone Medical.

Peer review information
Nature Reviews Nephrology thanks J. Bedke, R. Deberardinis, J. Hsieh and R. Weiss for their contribution to the peer review of this work.

Publisher's note