

Mitochondrial metabolic remodelling in response to genetic and environmental perturbations

Hollinshead, Kate; Tennant, Daniel

DOI:

[10.1002/wsbm.1334](https://doi.org/10.1002/wsbm.1334)

License:

Creative Commons: Attribution (CC BY)

Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Hollinshead, K & Tennant, D 2016, 'Mitochondrial metabolic remodelling in response to genetic and environmental perturbations', *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, vol. 8, no. 4, pp. 272-285. <https://doi.org/10.1002/wsbm.1334>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.



Mitochondrial metabolic remodeling in response to genetic and environmental perturbations

Kate E.R. Hollinshead and Daniel A. Tennant*

Mitochondria are metabolic hubs within mammalian cells and demonstrate significant metabolic plasticity. In oxygenated environments with ample carbohydrate, amino acid, and lipid sources, they are able to use the tricarboxylic acid cycle for the production of anabolic metabolites and ATP. However, in conditions where oxygen becomes limiting for oxidative phosphorylation, they can rapidly signal to increase cytosolic glycolytic ATP production, while awaiting hypoxia-induced changes in the proteome mediated by the activity of transcription factors such as hypoxia-inducible factor 1. Hypoxia is a well-described phenotype of most cancers, driving many aspects of malignancy. Improving our understanding of how mitochondria change their metabolism in response to this stimulus may therefore elicit the design of new selective therapies. Many of the recent advances in our understanding of mitochondrial metabolic plasticity have been acquired through investigations of cancer-associated mutations in metabolic enzymes, including succinate dehydrogenase, fumarate hydratase, and isocitrate dehydrogenase. This review will describe how metabolic perturbations induced by hypoxia and mutations in these enzymes have informed our knowledge in the control of mitochondrial metabolism, and will examine what this may mean for the biology of the cancers in which these mutations are observed.

© 2016 The Authors. *WIREs Systems Biology and Medicine* published by Wiley Periodicals, Inc.

How to cite this article:

WIREs Syst Biol Med 2016. doi: 10.1002/wsbm.1334

INTRODUCTION

Mitochondria are a ubiquitous feature of eukaryotic cells, thought to have been incorporated as a core component of our cellular machinery at approximately the same time as the increase in atmospheric oxygen levels, around 1.5 billion years ago.¹ They are hypothesized to be the foundation of many aspects of metazoan phenotype, including the ability to differentiate and our considerable metabolic plasticity. Mitochondria are also unique within

the eukaryotic cell, consisting of a double lipid bilayer, a specific lipid component (cardiolipin) not otherwise found in the cell, and their own DNA. As metabolic hubs of the cell, mitochondria integrate the use of diverse carbon sources, including sugars and their downstream metabolites, lipids, amino acids, and ketone bodies for the generation of cellular energy (ATP). They are also central to the conversion of one carbon source into another, permitting the synthesis of lipids from sugars and glucose from amino acids. Without them, cells would be forced to rely on exogenous nutrient sources for processes such as cell repair and proliferation.

The function of mammalian mitochondria is greatly dependent on an oxygenated microenvironment and a highly regulated complement of metabolic enzymes, some of which are unique within the

*Correspondence to: d.tennant@bham.ac.uk

Institute of Metabolism and Systems Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK

Conflict of interest: The authors have declared no conflicts of interest for this article.

cell. In the early 1900s, Dr Otto Warburg made the observation that cancer cells produce significant lactate in the presence of oxygen, which led him to the assertion that mitochondrial dysfunction was a root cause of all cancers.^{2,3} Although this was later shown not to be the case as a generalized mechanism, it has not escaped the attention of cancer biologists more recently that mitochondrial dysfunction is often observed in cancer.^{4–6} However, the role of this dysfunction—whether a driver, a necessary supporter, or just a side act—is not always clear. This review will outline from a cancer perspective how mitochondrial function is known to be affected by oxygen tension, and the effect of mutations in some of the metabolic enzymes within and associated with the mitochondria that have been shown to play a role in the formation or phenotype of some cancers.

HYPOXIA AND MITOCHONDRIAL FUNCTION

As tumors grow from a single transformed cell into a cell mass, they create a significant demand for glucose and oxygen that outweighs supply. The partial pressure of oxygen therefore decreases within the tumor, resulting in a reduced ability of cells to produce ATP through oxidative phosphorylation. In turn, the repression of respiration on glycolysis is lost, and glycolytic ATP production increases to compensate.⁷ Decreased respiration also results in a reduction in the rate of NADH oxidation by complex I of the respiratory chain, leading to an increase in the NADH:NAD⁺ ratio in the mitochondria.^{8,9} This increase inhibits the reducing potential of the cytosolic NADH produced in glycolysis from being transferred into the mitochondria through the malate–aspartate shuttle. As a result, the NADH must be oxidized in the cytosol to permit continued ATP production through glycolysis by the reduction of pyruvate to lactate. Without any compensatory steps, the increase in the NADH:NAD⁺ ratio in the mitochondria means that in hypoxia, the NADH-producing reactions of the tricarboxylic acid (TCA) cycle are inhibited (Figure 1), reducing flux through the pathway, and thereby inhibiting oxidative anabolic metabolism from mitochondrial carbon sources. As a result, cells would be forced into a nonproliferative, energy-sparing phenotype—producing most ATP through glycolysis and downregulating processes that demand significant energy and macromolecules such as protein synthesis. Hypoxia has previously been shown to elicit an autophagic

response,^{8,10} which could supply some cellular macromolecules, but without a degree of mitochondrial remodeling, continued anabolism in these conditions is not possible.⁸ Instead, as a measure of compensation, the TCA cycle is split into a number of linear pathways under these conditions.

In diving mammals and marine invertebrates, which can survive underwater for significant periods, it has been noted that succinate accumulates in the peripheral blood while the animal is in hypoxic conditions.¹¹ Indeed, the hypoxic accumulation of succinate has been observed in mammalian tissues, and has been attributed to the mitochondrial reduction of fumarate by succinate dehydrogenase (SDH) to continue NADH oxidation by complex I^{12,13}—a functional reversibility that has been known for over 50 years.¹⁴ This means that mitochondrial ATP synthesis as well as glycolysis can be maintained with reduced lactate production, and therefore with a longer period before acidosis, decreasing the need to resurface to service the oxygen debt. The fumarate reductase activity of SDH has now been described in both normal ischemic and hypoxic cancer cells, showing that this is a universal phenotype of mammalian mitochondria.^{13,15} In these conditions, a source of mitochondrial fumarate is required. It has been suggested in ischemic cells that both import of aspartate and reversal of part of the TCA cycle and the use of the purine nucleotide cycle can provide sources of fumarate.¹³ The purine nucleotide cycle is an extra-mitochondrial cycle that is required to aminate inosine monophosphate (IMP) to form adenosine monophosphate through the activities of adenylosuccinate synthetase (ADSS) and adenylosuccinate lyase (ADSL). The amine group is provided by aspartate, which as a result is converted to fumarate (Figure 1), and the AMP can be used in energy metabolism or to complete the cycle through the activity of AMP deaminase (AMPD), which evolves ammonium and IMP.

The metabolism of hypoxic mitochondria in a cellular context has also been shown to produce higher levels of reactive oxygen species (ROS) than in normoxia.¹⁶ Reduced complex I and III have been shown to donate electrons to available oxygen, forming the highly reactive superoxide radical, which can oxidize available proteins, lipids, and DNA in the nearest vicinity or be dismutated to hydrogen peroxide, which is able to travel further and oxidize molecules in more of the cellular space.⁹ There are a number of mitochondrial enzymes that are highly susceptible to oxidant-mediated inactivation, including complex I, aconitase, and α -ketoglutarate dehydrogenase (α KGDH).^{17–20} Interestingly, the inactivation of

mitochondrial metabolism through the phosphorylation and inhibition of the E1 α subunit of the pyruvate dehydrogenase complex (PDC), which oxidatively decarboxylates pyruvate to form acetyl CoA, a very important donor of mitochondrial acetyl groups to not only the epigenetic machinery but also the TCA cycle. The upregulation of PDK1 and consequent inhibition of the PDC has been shown as vital in maintaining cell viability in hypoxia, through the reduction of ROS production and stabilization of ATP levels.²⁵ These phenotypes could be designed to avoid activation of cellular senescence pathways in hypoxia, as suppression of the PDC by oncogene activation has recently been shown as critical in suppressing oncogene-induced senescence (OIS).²⁷ Indeed, further evidence of the use of metabolic remodeling in hypoxia to avoid ROS-mediated senescence arises from evidence that inhibition of the increased glycogen metabolism observed in hypoxia results in senescence through increased ROS production.²⁸ It is therefore likely that the inhibition of the PDC by HIF1-induced PDK1 upregulation reduces ROS-induced senescence in hypoxia. However, loss of pyruvate oxidation can also result in a truncated TCA cycle, in which the oxaloacetate (OAA) produced by glutamine oxidation cannot be used to produce citrate without another source of mitochondrial acetyl CoA. It is possible that the pool of acetyl CoA is maintained through the β oxidation of fatty acids in hypoxia, but if the mitochondrial aconitase is also inhibited in these conditions, through the increased production of ROS in hypoxia, this is unlikely.

Loss of PDC activity has been shown to result in a change in the citrate to α KG ratio in the cell, which has been suggested to be a prerequisite for a change in the way in which glutamine is metabolized: inducing an increase in the use of reductive carboxylation of this amino acid²⁹ (Figure 1). This is a potentially important pathway in hypoxia, as pyruvate oxidation is inhibited by HIF1-dependent upregulation of PDK1, producing a deficit of the metabolites in the proximal portion of the TCA cycle (citrate and isocitrate). Indeed, as previously suggested, a reduction in oxygen tension may also be limiting for oxidation of glutamine, making reductive carboxylation more relied upon for anabolic metabolism.³⁰ The enzymes responsible for the reductive metabolism of glutamine can be found both in the mitochondrial matrix and the cytosol (ACO2 and IDH2; ACO1 and IDH1, respectively), and may be able to compensate for this deficit in these conditions.^{29,31} It is therefore consistent that reductive carboxylation has been suggested as an essential pathway for cancer cell

survival in hypoxia or for those with defective oxidative mitochondrial machinery.^{30,32–34} Although it is theoretically possible that both pathways contribute to this reductive activity, the cytosolic compartment is much more easily accessible to exogenous glutamine than the mitochondrial matrix, as the latter requires transport of the precursor (glutamine/glutamate/ α KG) into the matrix and the transport of the resulting citrate out. It is unknown how hypoxia may alter the activity of the necessary transporters, which requires further study.

Further HIF1-dependent modification of mitochondrial metabolism in hypoxia has been reported through subunit switching of complex IV (cytochrome c oxidase, COX) of the respiratory chain. COX is a homodimer with 13 subunits per monomer, which sits within the mitochondrial inner membrane.³⁵ In common with most complexes of the respiratory chain, the subunits of COX are encoded by genes in both the mitochondrial and nuclear DNA. COX4, one of the centrally situated subunits of the complex, is encoded by the nuclear genome with two paralogues: COX4-1 and COX4-2.³⁶ In both yeast (in which this subunit is known as Cox5) and some human tissues, reduction in environmental oxygen tension elicits a repression of the expression of the 'a' or '1' subunit and an increase in expression of the 'b' or '2' subunit.^{37–39} In cancer cells, it was shown that this switching was brought about by the HIF1-induced expression of the COX4-2 transcript, alongside increased expression of the mitochondrial LON protease, which degraded the COX4-1 subunit.³⁷ From studies in yeast, it is thought that expression of the 'b' subunit may permit a more rapid transfer of electrons to COX1 from the heme moiety.^{40,41} The study by Fukuda et al. in cancer cells suggests that this may reduce mitochondrial ROS production in hypoxia.³⁷ However, the general overall effect of this on hypoxic mitochondrial biology is not entirely clear, as it appears that COX4-2 expression may only be regulated by hypoxia in a subset of tissues (lung and liver).³⁷

The major hydroxylase that determines HIF1 α stability is PHD2,⁴² suggesting that the majority of HIF1-mediated changes in mitochondrial metabolism are dependent on the inhibition of PHD2 in hypoxia. However, recent studies investigating the PHD1^{-/-} mouse have shown that hypoxia may induce other changes in mitochondrial metabolic activity, independently of HIF1.^{43,44} These changes have not yet been adequately studied and represent a significant gap in our knowledge of how hypoxia remodels cellular metabolism in general.

MUTATIONS OF MITOCHONDRIAL (AND ASSOCIATED) METABOLIC ENZYMES IN CANCER

The identification of cancer-associated mutations in the metabolic enzymes such as fumarate hydratase (FH), SDH, and isocitrate dehydrogenase (IDH) has demonstrated that altered mitochondrial metabolism is sufficient to transform some cell types.⁴⁵ *FH* and *SDH* are *bone fide* tumor suppressor genes, inactivating mutations in which have been identified as causative in cancers such as renal clear-cell carcinoma and paraganglioma.^{46–50} Mutations in *IDH1* and *IDH2* are common in a variety of diverse cancers, including adult gliomas^{51,52} and acute myelogenous leukemia (AML),^{53,54} but unlike *FH* and *SDH*, are genetic oncogenes. A single hit, which is both loss of function and gain of function, is only ever observed in these genes. In both gliomas and AML, mutations in *IDH* genes have been suggested as a very early, and perhaps founder mutations, although in neither disease does *IDH* mutation appear to be able to induce transformation on its own.^{55–58} What is common to all three genes is that they are either situated directly within the mitochondrial matrix (*SDH*, *FH*, and *IDH2*) or their metabolic activity directly affects that of the mitochondrion (*IDH1*). In addition, the cancer-associated mutations observed have all been reported to interact with the HIF system; in the case of *FH* and *SDH* mutations, these unequivocally result in HIF α stabilization, whereas in the case of *IDH1* and *IDH2*, it appears that mutation may destabilize hypoxic HIF α stabilization. In the latter case, it would appear that the biological outcome of this is not yet clear. Mutations in these enzymes therefore have a dual outcome on mitochondrial metabolic activity: they both alter HIF1 activity and they directly alter metabolism.

Succinate Dehydrogenase

SDH is a highly conserved heterotetrameric complex encoded entirely by the nuclear genome. It is responsible for the oxidation of succinate to fumarate in the TCA cycle and the transfer of electrons to ubiquinone, transfer of which is required by complex III to pump protons. Complex II itself is unique in the respiratory chain as the only complex without proton pumping function. The complex consists of two subunits (SDHA and SDHB), which protrude into the mitochondrial matrix, and two hydrophobic subunits (SDHC and SDHD), which anchor the catalytic components to the inner mitochondrial membrane and produce the binding site for ubiquinone.⁵⁹ Mutations

in *SDHD* were the first to be identified as causative in patients with hereditary paragangliomas and later pheochromocytomas, rare neuroendocrine neoplasms of the chromaffin tissue of the adrenal medulla and the parasympathetic tissue of the head and neck paraganglioma, respectively.⁵⁰ Since the initial discovery of *SDHD* mutations in 2000, mutations in *SDHB* and *SDHC*, and more recently, *SDHA* and *SDHAF2* (encoding the protein responsible for FAD incorporation into SDH) have been identified with the same syndrome.^{47–49,60} These mutations are found as heterozygous germline mutations, which means that loss of protein function and neoplastic transformation develops as a result of loss of heterozygosity, resulting in the complete loss of enzyme function by a second ‘hit.’⁶¹ Inactivating mutations of SDH subunits have since been reportedly associated with other tumor types including gastrointestinal stromal tumors,⁶² renal cell carcinoma,^{63,64} and neuroblastoma.^{65,66} Interestingly, mutations of SDHB predispose to a more malignant and aggressive form of paraganglioma than those of the other subunits, despite the apparent similarities in both functional and downstream phenotypic consequences of this mutation. It is apparent from this clinical perspective that we do not fully understand the role of SDHB in SDH function.

SDH is one of the few enzymes in the TCA cycle that are unique to the mitochondrion, making this compartment the major source of succinate, and the only one in which it can be further metabolized within the mammalian cell. Loss of SDH function through mutation in any one of the subunits results in loss of dehydrogenase activity, succinate accumulation within the cell, and excretion into the extracellular space. The significant increase in succinate concentrations leads to the inhibition of all members of the α KG-dependent dioxygenase family that have been reported, including histone and DNA demethylases and the PHDs. This produces a hypermethylator phenotype of the chromatin⁶⁷ as well as a pseudohypoxic phenotype through the stabilization of HIF in the presence of oxygen.⁶⁸ This has the significant consequence of changing mitochondrial activity to a more ‘hypoxia-like’ state through HIF1 signaling. In addition, it has been suggested that SDH-deficient mitochondria may generate more superoxide, resulting in additional enzyme inactivation as previously described for hypoxia.⁶⁹

Although the loss of SDH activity within the mitochondria produces another form of truncated TCA cycle (Figure 2), the significant production of succinate shows that this new TCA cycle activity is used by cells. However, until recently, the models

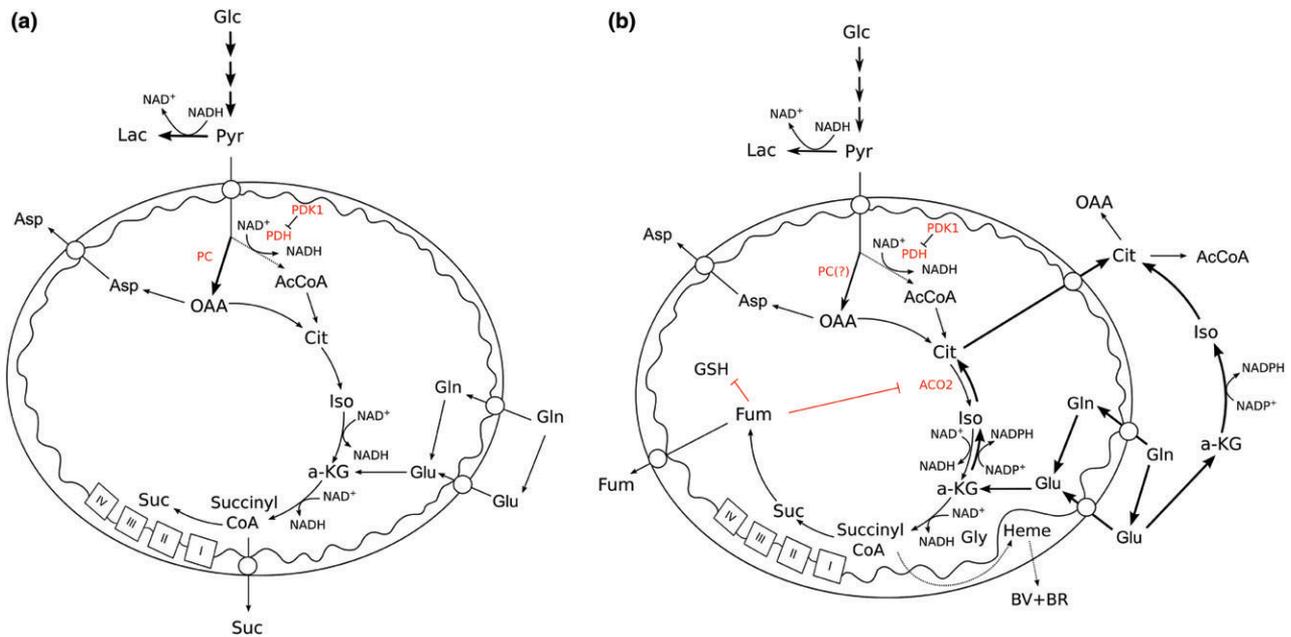


FIGURE 2 | Succinate dehydrogenase (SDH) (a) and fumarate hydratase (FH) (b) mutation-mediated alterations in mitochondrial metabolism. Metabolic pathways that have been shown to be used in cells or tumors containing these mutations are shown. Pathways that are likely to be used, but have not yet been shown are marked with '?' Abbreviations: AcCoA, acetyl CoA; ACO2, mitochondrial aconitase; a-KG, α -ketoglutarate; Asp, aspartate; BR, bilirubin; BV, biliverdin; Cit, citrate; Fum, fumarate; Glc, glucose; Gln, glutamine; Glu, glutamate; GSH, reduced glutathione; Gly, glycine; Lac, lactate; OAA, oxaloacetate; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; Pyr, pyruvate; Suc, succinate.

available for interrogation of this activity were lacking. Two recent studies have described aspects of the metabolic remodeling that mitochondria undergo in the absence of Sdhb.^{70,71} As would be expected from the stabilization of HIF1, Sdhb-deficient cells were shown to have significantly inhibited pyruvate oxidation, but pyruvate was still metabolized by the mitochondria through carboxylation by pyruvate carboxylase (PC). Indeed, this was found to be critical for continued Sdhb-deficient cell proliferation both *in vitro* and *in vivo*^{70,71} most likely due to the loss of aspartate synthesis in these cells (Figure 2)—an amino acid required for a number of key anabolic pathways including protein and nucleotide synthesis. Importantly, those cells with wild-type SDH activity were able to tolerate siRNA-induced PC deficiency and maintain their proliferative capability through continued synthesis of aspartate through the oxidative TCA cycle. The continued production of succinate by SDH-deficient cells indicates the ability of their mitochondria to retain some NADH oxidative activity and therefore continued oxidative ATP production. Indeed, it appears that glutamine carbons may represent the major carbon source for the succinate produced,^{70,71} suggesting that it is the activity of α KGDH that delivers significant NAD⁺ reducing

activity in the absence of SDH. However, important questions remain—one of the most intriguing being whether cells and tumors bearing loss of function of SDH through mutations in other subunits show the same metabolic phenotype. This is key, as *SDHB*-mutated tumors are the only SDH-mutated tumors result in malignant tumors. One of the few studies to examine the differential effects of deficiencies of different SDH subunits suggested that RNAi-mediated reduction in the expression of SDHB led to increased mitochondrial ROS production, while this was not recapitulated by similar reduction of SDHA.⁷² However, another study has suggested that SDHB deficiency does not result in detectable induction of ROS production.⁷³ It therefore remains unclear as to the basis of the malignancy in SDHB-deficient tumors, and further research is required.

Fumarate Hydratase

FH, encoding the proximal downstream enzyme to SDH, is another mitochondrial tumor suppressor gene, the loss of which leads to hereditary leiomyomatosis and renal-cell cancer (HLRCC).⁴⁶ FH-deficient leiomyomas (a benign neoplasm) are highly penetrant, while RCC occurs slightly less

frequently.⁷⁴ However, the RCC that develop are highly aggressive, leading to the early generation of distant metastases,⁷⁴ similar to SDHB-deficient tumors. Although a single gene, processing at the mitochondrion allows the protein to be distributed in both cytosolic and mitochondrial compartments.⁷⁵

FH is responsible for the hydration of fumarate to form malate, which can then either be oxidized by malate dehydrogenase to form NADH and oxaloacetate or oxidatively decarboxylated by malic enzyme to form pyruvate and NADPH (Figure 1). Deficiencies in FH lead to high steady-state concentrations of fumarate in the cell and surrounding extracellular space. In parallel to succinate, fumarate has been shown to competitively inhibit α KG-dependent dioxygenase enzymes, resulting in a pseudohypoxic transcriptome through HIF α stabilization⁷⁶ and a hypermethylator phenotype.^{76,77} However, contrary to initial thoughts, now there is evidence to suggest that stabilization of HIF is not the driver of tumorigenesis in FH-deficient tumors, and that a different candidate transcription factor—the nuclear-related factor 2 (NRF2) pathway—may instead be involved.^{78,79}

In addition to the truncation of the TCA cycle and increase in reductive glutamine metabolism that occurs as a result of loss of FH function,³⁴ fumarate is also a product of two other metabolic pathways (in contrast to succinate): the urea and purine nucleotide cycles as well as tyrosine metabolism. The high concentrations of fumarate produced in FH-deficient cells have been shown to result in reversal of part of the urea cycle, producing argininosuccinate and disrupting arginine metabolism within FH-deficient cells.^{80,81} In addition, fumarate has been shown to react with enzymes and other intracellular metabolites in a process known as succination.^{82–84} This posttranslational modification has been shown to inhibit mitochondrial aconitase activity in FH-deficient cells, resulting in further truncation of the TCA cycle⁸² (Figure 2). In addition, succination of reduced glutathione (GSH) to form succinyl-GSH has also been demonstrated, which results in the depletion of the intracellular antioxidant pool and senescence.⁸³ Importantly, this sheds further light on the mutational hits required for an FH-deficient lesion to become malignant, and suggests that upregulation of NRF2-dependent antioxidant pathways, as previously described, is likely to be important to compensate for the reduced GSH pool. In mice, inactivation of cell cycle inhibitors, such as p21, has been shown to be important for tumor development in *Fh1*^{-/-}

kidneys.⁸³ In addition, it is highly likely that other exposed thiol groups in mitochondrial-associated enzymes are potential targets of succination, and therefore other metabolic perturbations may be present in these cells.

A bioinformatic analysis of *Fh1*-deficient cells highlighted a mitochondrial metabolic ‘bypass’ required for viability.⁸⁵ Frezza et al. showed that *Fh1*-deficient cells continue to oxidize glutamine through α KGDH (reducing NAD⁺ for oxidation by complex I of the respiratory chain) but direct a proportion of the succinyl CoA produced by the subsequent enzymatic step of the TCA cycle, succinyl CoA ligase, through the heme biosynthetic pathway⁸⁵ (Figure 2). This pathway, which starts and finishes in the mitochondrial matrix, uses four molecules each of succinyl CoA and glycine to produce heme, so appears to be a very efficient means of removing the excess carbon from the cell. However, Frezza et al. showed that the degradation of heme was also required, as pharmacological block or knockdown of heme oxygenase, the first enzyme in the degradation of heme, resulted in loss of cell viability.⁸⁵ Heme degradation releases iron and CO, producing biliverdin and then bilirubin. Interestingly, the use of this pathway may not only be due to a requirement to excrete excess TCA cycle carbon. It has been noted that both biliverdin and bilirubin can act as antioxidants, capable of quenching superoxide—perhaps a useful function in cells deficient for GSH.^{86,87} Although the cellular localization of heme oxygenase is not clear, it is possible that release of the CO in proximity to the respiratory chain could have consequences for continued electron flow and therefore NADH-oxidizing activity. Importantly, the synthesis of heme also required considerable glycine (1:1 with succinyl CoA), which is likely to make cells highly dependent on the glycine synthetic pathways, controlled by serine hydroxymethyltransferase (SHMT) 1 and 2. As this part of the pathway occurs in the mitochondrial matrix, SHMT2, the matrix isozyme may be key in FH-deficient cell function.

The bioinformatics screen performed on FH-deficient cells also highlighted other enzymes that may be synthetic lethal with loss of *Fh1*.⁸⁵ Of particular note is the mention of PC, which was later shown to be synthetic lethal with loss of *Sdhb*,^{70,71} glutaminase (Gln), which can be understood through the cells requirement for this amino acid for mitochondrial oxidative activity, and SDH. This last prediction is intriguing, but no further data are yet available to show whether this is a true synthetic lethal combination with *Fh1* deficiency.

Isocitrate Dehydrogenase

IDH enzymes are members of the β -decarboxylating dehydrogenase family that catalyze the oxidative decarboxylation of isocitrate to α KG and CO_2 . Mammalian cells express three IDH isoforms (1, 2, and 3) encoded by five genes (*IDH1*, 2, *3A/3B/3G*) in the nuclear genome. IDH1 and IDH2 are reversible, highly homologous (>70%) homodimeric NADP⁺-dependent enzymes. IDH3 is an irreversible heterotetrameric NAD⁺-dependent enzyme, both genetically and structurally unrelated to IDH1 and IDH2. Interestingly, mutations only in *IDH1* and *IDH2* have thus far been linked to cancer. The presence of recurrent somatic point mutations in *IDH1* was first identified in 12% of all adult glioblastoma tumors by high-throughput DNA sequencing.⁵¹ Deep sequencing further revealed that mutated *IDH1* and *IDH2* occur in a high proportion (>70%) of low-grade (grade II–III) astrocytomas and oligodendrogliomas and secondary glioblastoma.^{51,52,88,89} Since these initial reports, mutations in *IDH1* and *IDH2* have also been found in other diverse cancers including 16–17% of patients with AML,⁵³ 20% of patients with angioimmunoblastic T-cell lymphomas,⁹⁰ and more rarely in cholangiocarcinomas,⁹¹ breast carcinomas,⁹² acute lymphoblastic leukemias,⁹³ prostate cancer,⁹³ osteosarcoma,⁹⁴ and others.^{53,91–93,95–97} Interestingly, and unlike IDH mutations in glioma which are almost entirely in *IDH1*, AML have been shown to exhibit an approximately equal frequency of *IDH1* and *IDH2* mutations.^{53,54}

IDH1-mutated gliomas have all been reported to have a single-point mutation within the codon for Arg132. It appears that the removal of the arginine, rather than the substitution of a specific residue, is the critical feature of this mutation, although the most frequent replacement observed is a histidine residue (R132H).^{51,52,93,98} Mutations in *IDH2* in glioma occur at a much lower frequency (0–6%) than *IDH1*⁸⁸ in the paralogous amino acid residue—R140—but also at R172. These residues are key in the activity of IDH enzymes: Arg132 and Arg172 in IDH1 and Arg140 and Arg172 in IDH2 form hydrophilic hydrogen bonds with the α -carboxyl and β -carboxyl groups of isocitrate to facilitate isocitrate binding in the enzyme active site.^{99,100} Mutations in these residues decrease binding affinity for isocitrate and increase binding affinity for NADPH, leading to the reduction of α KG by NADPH and the release of the (R) enantiomer of 2-hydroxyglutarate [(R)-2HG].¹⁰¹ The (R)-2HG produced by the mutant IDH enzymes accumulates in cells to extremely high

millimolar concentrations, disrupting a number of cellular functions.¹⁰⁰ However, it is only the concentration and not the production of (R)-2HG which is nonphysiological in *IDH*-mutated tumors, as it is also produced as a minor product by α KG reductase enzymes and is oxidized back to α KG by 2HG dehydrogenases.¹⁰² Interestingly, cells expressing mutant IDH1 produce less (R)-2HG than those with IDH2 mutations.¹⁰³ Moreover, (R)-2HG expression by mutant IDH1 is enhanced by the co-expression of wild-type IDH1, leading to the formation of heterodimers.^{100,103} Loss of the wild-type IDH1 expression results in a dramatic decrease in (R)-2HG production,¹⁰⁴ suggesting either that the presence of wild-type IDH1 increases the enzymatic activity of mutant IDH1 toward α KG or that a cycling activity (redox neutral) permits a higher production of (R)-2HG with a more stable cell phenotype.

Owing to the structural similarity of (R)-2HG to α KG, the effect of *IDH* mutations on members of the α KG-dependent dioxygenase family of enzymes has also been studied. Much like SDH- and FH-mutated tumors, IDH-mutated cells and tumors have been shown to exhibit a hypermethylator phenotype through the inhibition of the ten-eleven translocation (TET) family of DNA demethylases and members of the Jumonji C-domain histone demethylases through the competitive inhibition of α KG binding by (R)-2HG.^{105–109} However, unlike *SDH* and *FH* mutations, the effect of (R)-2HG on PHD activity has been the subject of some controversy. Although an initial report suggested that the PHD enzymes were inhibited by (R)-2HG production resulting in increased HIF1 α protein,¹¹⁰ the evidence that *IDH1* mutant cells and tumors actively suppress HIF α stabilization through activation of the PHDs is now more compelling. The precise means by which (R)-2HG results in increased PHD enzyme activity, however, is still not clear. It has been suggested to be through direct use of only the (R) enantiomer by the PHDs as a substrate^{109,111} or through nonenzymatic oxidation of 2HG into α KG, therefore acting as a source of the normal substrate.¹¹² Consistent with either mechanism, IDH mutant cells display decreased HIF activation compared with their wild-type counterparts.¹⁰⁹ Mutations in *IDH1* and *IDH2*, in both glioma and AML, occur early in tumor development and are thought to be mutually exclusive,^{52,113} suggesting that either isoform is sufficient to confer a predisposition to or permit transformation when mutated. The IDH1 and IDH2 isozymes differ only in their localization, yet although they are equally prevalent in patients with *IDH*-mutated AML, gliomas with these

mutations are almost always *IDH1*-mutated. This discrepancy has not yet been explored fully, but it is possible that the environment in which each disease develops (such as the extent and severity of hypoxia) and/or a lack of suitable mitochondrial metabolic plasticity may play a role in determining why *IDH2* mutations are not observed often in gliomas.

Gliomas are highly hypoxic tumors.^{114–116} As outlined above, it has previously been shown that hypoxic cells may rely more on reductive glutamine metabolism to produce citrate (and thereby lipids) than normoxic cells.³⁰ Both *IDH1* and 2 have potential roles in hypoxia-induced reductive carboxylation,^{29,31} suggesting that this pathway may be perturbed in *IDH1*-mutated gliomas. Given that the cytosolic pathway has been shown to be important in hypoxic reductive carboxylation for lipogenesis,^{30,32–34} and that gliomas appear to ‘favor’ *IDH1* mutations, there may be a requirement for *IDH2* wild-type activity in hypoxic *IDH1* mutant gliomas. However, a recent paper also showed that *IDH1*-mutated cells retained more of their oxidative TCA cycle metabolism than their wild-type counterparts.¹¹⁷ Under hypoxic conditions, where *IDH1* wild-type cells increased their dependence on reductive carboxylation of glutamine, *IDH1* mutant cells increased their use of oxidative decarboxylation, most likely retaining oxidative ATP generation in these conditions. This is consistent with the evidence that (R)-2HG inhibits hypoxia-induced stabilization of HIF1 α ,¹⁰⁹ and therefore *IDH1*-mutated cells may not exhibit the HIF-dependent metabolic response to hypoxia, including changes in the activity of the PDC and complex IV. However, continued oxygen use in hypoxia is likely to create highly challenging conditions in an *IDH1*-mutated tumor, as it is likely to further exacerbate the hypoxic microenvironment, potentially leading to increased areas of necrosis. It is important to note that *IDH1* mutant gliomas demonstrate improved overall survival compared to their wild-type counterparts,⁵² which may well be in some part due to their apparent inability to modify their

mitochondrial metabolism in response to hypoxia. Interestingly, *IDH2*-mutated cells were shown not to exhibit the same alteration in the hypoxia-mediated mitochondrial metabolic phenotype.¹¹⁷ These data may shed more light on the metabolic differences induced by *IDH1* and *IDH2* mutations and therefore their different prevalence in glioma—a highly hypoxic type of tumor.

FUTURE PERSPECTIVES

It is thought that the ancestors of mitochondria, perhaps some type of Rhodobacteria, were organisms with a highly plastic metabolism, able to make ATP both in the presence and absence of oxygen. Although our mitochondria are removed from their predecessors by almost 2 billion years, the mutations that we observe in cancer and studies of other mammals have shown us that they retain many of these characteristics. It is also important to note that studies of mitochondrial metabolism are often centered around only one small aspect of their metabolism—the TCA cycle—and ignore other highly significant metabolic pathways for which mitochondria are critical. While classically being considered the TCA ‘cycle,’ it is becoming increasingly clear through studies on perturbed mitochondrial systems that it in fact consists of multiple interconnecting metabolic pathways that permit crosstalk between the mitochondrion and the cytosol. It is only through studies on tumors—highly proliferative cell systems—that we find that the cycle itself is dispensable, where the use of only some specific linear portions of the TCA cycle is required to fulfill the anabolic requirements without significant difficulty. However, it is in adapting to the truncated nature of the TCA cycle that *SDH*-, *FH*-, and *IDH*-mutated and hypoxic cells have been shown to become highly dependent on one or more pathways for their survival. These metabolic vulnerabilities therefore represent attractive targets for future therapeutic development.

ACKNOWLEDGMENTS

We thank Dr Katarina Klučkova for her critical reading of the manuscript. KERH is supported through a CASE award studentship from the BBSRC and AstraZeneca.

REFERENCES

1. Embley TM, Martin W. Eukaryotic evolution, changes and challenges. *Nature* 2006, 440:623–630.
2. Warburg O. On the origin of cancer cells. *Science* 1956, 123:309–314.

3. Warburg O. Metabolism of tumours. *Biochem Zeitschr* 1923, 142:317–333.
4. Guaragnella N, Giannattasio S, Moro L. Mitochondrial dysfunction in cancer chemoresistance. *Biochem Pharmacol* 2014, 92:62–72.
5. Chen EL. Mitochondrial dysfunction and cancer metastasis. *J Bioenerg Biomembr* 2012, 44:619–622.
6. Frezza C. The role of mitochondria in the oncogenic signal transduction. *Int J Biochem Cell Biol* 2014, 48:11–17.
7. Rodriguez-Enriquez S, Carreno-Fuentes L, Gallardo-Perez JC, Saavedra E, Quezada H, Vega A, Marin-Hernandez A, Olin-Sandoval V, Torres-Marquez ME, Moreno-Sanchez R. Oxidative phosphorylation is impaired by prolonged hypoxia in breast and possibly in cervix carcinoma. *Int J Biochem Cell Biol* 2010, 42:1744–1751.
8. Frezza C, Zheng L, Tennant DA, Papkovsky DB, Hedley BA, Kalna G, Watson DG, Gottlieb E. Metabolic profiling of hypoxic cells revealed a catabolic signature required for cell survival. *PLoS One* 2011, 6:e24411.
9. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J* 2009, 417:1–13.
10. Mazure NM, Pouyssegur J. Hypoxia-induced autophagy: cell death or cell survival? *Curr Opin Cell Biol* 2010, 22:177–180.
11. Hochachka PW, Owen TG, Allen JF, Whittow GC. Multiple end products of anaerobiosis in diving vertebrates. *Comp Biochem Physiol* 1974, 50B:17–22.
12. Hoberman HD, Prosky L. Evidence of reduction of fumarate to succinate in perfused rat liver under conditions of reduced O₂ tension. *Biochim Biophys Acta* 1967, 148:392–399.
13. Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord EN, Smith AC, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature* 2014, 515:431–435.
14. Sanadi DR, Fluharty AL. On the mechanism of oxidative phosphorylation. VII. The energy-requiring reduction of pyridine nucleotide by succinate and the energy-yielding oxidation of reduced pyridine nucleotide by fumarate. *Biochemistry* 1963, 2:523–528.
15. Tomitsuka E, Kita K, Esumi H. Regulation of succinate-ubiquinone reductase and fumarate reductase activities in human complex II by phosphorylation of its flavoprotein subunit. *Proc Jpn Acad Ser B Phys Biol Sci* 2009, 85:258–265.
16. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci USA* 1998, 95:11715–11720.
17. Gardner PR. Superoxide-driven aconitase FE-S center cycling. *Biosci Rep* 1997, 17:33–42.
18. Riobo NA, Clementi E, Melani M, Boveris A, Cadenas E, Moncada S, Poderoso JJ. Nitric oxide inhibits mitochondrial NADH:ubiquinone reductase activity through peroxynitrite formation. *Biochem J* 2001, 359:139–145.
19. Humphries KM, Yoo Y, Szweda LI. Inhibition of NADH-linked mitochondrial respiration by 4-hydroxy-2-nonenal. *Biochemistry* 1998, 37:552–557.
20. Andersson U, Leighton B, Young ME, Blomstrand E, Newsholme EA. Inactivation of aconitase and oxoglutarate dehydrogenase in skeletal muscle in vitro by superoxide anions and/or nitric oxide. *Biochem Biophys Res Commun* 1998, 249:512–516.
21. Semenza GL. Hypoxia-inducible factor 1 (HIF-1) pathway. *Sci STKE* 2007, 2007:cm8.
22. Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. *Nat Rev Mol Cell Biol* 2004, 5:343–354.
23. Maxwell PH, Pugh CW, Ratcliffe PJ. The pVHL-hIF-1 system. A key mediator of oxygen homeostasis. *Adv Exp Med Biol* 2001, 502:365–376.
24. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, et al. Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. *Science* 2001, 292:468–472.
25. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 2006, 3:177–185.
26. Semenza GL. HIF-1: upstream and downstream of cancer metabolism. *Curr Opin Genet Dev* 2010, 20:51–56.
27. Kaplon J, Zheng L, Meissl K, Chaneton B, Selivanov VA, Mackay G, van der Burg SH, Verdegaal EM, Cascante M, Shlomi T, et al. A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. *Nature* 2013, 498:109–112.
28. Favaro E, Bensaad K, Chong MG, Tennant DA, Ferguson DJ, Snell C, Steers G, Turley H, Li JL, Gunther UL, et al. Glucose utilization via glycogen phosphorylase sustains proliferation and prevents premature senescence in cancer cells. *Cell Metab* 2012, 16:751–764.
29. Fendt SM, Bell EL, Keibler MA, Olenchock BA, Mayers JR, Wasylenko TM, Vokes NI, Guarente L, Vander Heiden MG, Stephanopoulos G. Reductive glutamine metabolism is a function of the α -ketoglutarate to citrate ratio in cells. *Nat Commun* 2013, 4:2236.
30. Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, Jewell CM, Johnson ZR, Irvine DJ, Guarente L, et al. Reductive glutamine metabolism by

- IDH1 mediates lipogenesis under hypoxia. *Nature* 2012, 481:380–384.
31. Gameiro PA, Yang J, Metelo AM, Perez-Carro R, Baker R, Wang Z, Arreola A, Rathmell WK, Olumi A, Lopez-Larrubia P, et al. In vivo HIF-mediated reductive carboxylation is regulated by citrate levels and sensitizes VHL-deficient cells to glutamine deprivation. *Cell Metab* 2013, 17:372–385.
 32. Wise DR, Ward PS, Shay JE, Cross JR, Gruber JJ, Sachdeva UM, Platt JM, DeMatteo RG, Simon MC, Thompson CB. Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α -ketoglutarate to citrate to support cell growth and viability. *Proc Natl Acad Sci USA* 2011, 108:19611–19616.
 33. Filipp FV, Scott DA, Ronai ZA, Osterman AL, Smith JW. Reverse TCA cycle flux through isocitrate dehydrogenases 1 and 2 is required for lipogenesis in hypoxic melanoma cells. *Pigment Cell Melanoma Res* 2012, 25:375–383.
 34. Mullen AR, Wheaton WW, Jin ES, Chen PH, Sullivan LB, Cheng T, Yang Y, Linehan WM, Chandel NS, DeBerardinis RJ. Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature* 2012, 481:385–388.
 35. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S. The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 Å. *Science* 1996, 272:1136–1144.
 36. Huttemann M, Kadenbach B, Grossman LI. Mammalian subunit IV isoforms of cytochrome c oxidase. *Gene* 2001, 267:111–123.
 37. Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell* 2007, 129:111–122.
 38. Trueblood CE, Poyton RO. Identification of REO1, a gene involved in negative regulation of COX5b and ANB1 in aerobically grown *Saccharomyces cerevisiae*. *Genetics* 1988, 120:671–680.
 39. Burke PV, Raitt DC, Allen LA, Kellogg EA, Poyton RO. Effects of oxygen concentration on the expression of cytochrome c and cytochrome c oxidase genes in yeast. *J Biol Chem* 1997, 272:14705–14712.
 40. Waterland RA, Basu A, Chance B, Poyton RO. The isoforms of yeast cytochrome c oxidase subunit V alter the in vivo kinetic properties of the holoenzyme. *J Biol Chem* 1991, 266:4180–4186.
 41. Allen LA, Zhao XJ, Caughey W, Poyton RO. Isoforms of yeast cytochrome c oxidase subunit V affect the binuclear reaction center and alter the kinetics of interaction with the isoforms of yeast cytochrome c. *J Biol Chem* 1995, 270:110–118.
 42. Berra E, Benizri E, Ginouves A, Volmat V, Roux D, Pouyssegur, J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1 α in normoxia. *EMBO J* 2003, 22:4082–4090.
 43. Aragonés J, Schneider M, Van Geyte K, Fraisl P, Dresselaers T, Mazzone M, Dirx R, Zacchigna S, Lemieux H, Jeoung NH, et al. Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. *Nat Genet* 2008, 40:170–180.
 44. Schneider M, Van Geyte K, Fraisl P, Kiss J, Aragonés J, Mazzone M, Mairbaur H, De Bock K, Jeoung NH, Mollenhauer M, et al. Loss or silencing of the PHD1 prolyl hydroxylase protects livers of mice against ischemia/reperfusion injury. *Gastroenterology* 2010, 138:e1141–e1142.
 45. Gaude E, Frezza C. Defects in mitochondrial metabolism and cancer. *Cancer Metab* 2014, 2:10.
 46. Tomlinson IP, Alam NA, Rowan AJ, Barclay E, Jaeger EE, Kelsell D, Leigh I, Gorman P, Lamlum H, Rahman S, et al. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat Genet* 2002, 30:406–410.
 47. Burnichon N, Briere JJ, Libe R, Vescovo L, Riviere J, Tissier F, Jouanno E, Jeunemaitre X, Benit P, Tzagoloff A, et al. SDHA is a tumor suppressor gene causing paraganglioma. *Hum Mol Genet* 2010, 19:3011–3020.
 48. Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, Skoldberg F, Husebye ES, Eng C, Maher ER. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am J Hum Genet* 2001, 69:49–54.
 49. Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat Genet* 2000, 26:268–270.
 50. Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 2000, 287:848–851.
 51. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science* 2008, 321:1807–1812.
 52. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med* 2009, 360:765–773.
 53. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, Koboldt DC, Fulton RS, Delehaunty KD, McGrath SD, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med* 2009, 361:1058–1066.

54. Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Collier HA, Cross JR, Fantin VR, Hedvat CV, Perl AE, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting α -ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* 2010, 17:225–234.
55. Chaturvedi A, Araujo Cruz MM, Jyotsana N, Sharma A, Yun H, Gorlich K, Wichmann M, Schwarzer A, Preller M, Thol F, et al. Mutant IDH1 promotes leukemogenesis in vivo and can be specifically targeted in human AML. *Blood* 2013, 122:2877–2887.
56. Sasaki M, Knobbe CB, Munger JC, Lind EF, Brenner D, Brustle A, Harris IS, Holmes R, Wakeham A, Haight J, et al. IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics. *Nature* 2012, 488:656–659.
57. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H. IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am J Pathol* 2009, 174:1149–1153.
58. Watanabe T, Vital A, Nobusawa S, Kleihues P, Ohgaki H. Selective acquisition of IDH1 R132C mutations in astrocytomas associated with Li-Fraumeni syndrome. *Acta Neuropathol* 2009, 117:653–656.
59. Bardella C, Pollard PJ, Tomlinson I. SDH mutations in cancer. *Biochim Biophys Acta* 2011, 1807:1432–1443.
60. Hao HX, Khalimonchuk O, Schradars M, Dephoure N, Bayley JP, Kunst H, Devilee P, Cremers CW, Schiffman JD, Bentz BG, et al. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science* 2009, 325:1139–1142.
61. Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat Rev Cancer* 2005, 5:857–866.
62. Janeway KA, Kim SY, Lodish M, Nose V, Rustin P, Gaal J, Dahia PL, Liegl B, Ball ER, Raygada M, et al. Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. *Proc Natl Acad Sci USA* 2011, 108:314–318.
63. Ricketts C, Woodward ER, Killick P, Morris MR, Astuti D, Latif F, Maher ER. Germline SDHB mutations and familial renal cell carcinoma. *J Natl Cancer Inst* 2008, 100:1260–1262.
64. Vanharanta S, Buchta M, McWhinney SR, Virta SK, Peczkowska M, Morrison CD, Lehtonen R, Januszewicz A, Jarvinen H, Juhola M, et al. Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am J Hum Genet* 2004, 74:153–159.
65. Cascon A, Landa I, Lopez-Jimenez E, Diez-Hernandez A, Buchta M, Montero-Conde C, Leskela S, Leandro-Garcia LJ, Leton R, Rodriguez-Antona C, et al. Molecular characterisation of a common SDHB deletion in paraganglioma patients. *J Med Genet* 2008, 45:233–238.
66. Armstrong R, Greenhalgh KL, Rattenberry E, Judd B, Shukla R, Losty PD, Maher ER. Succinate dehydrogenase subunit B (SDHB) gene deletion associated with a composite paraganglioma/neuroblastoma. *J Med Genet* 2009, 46:215–216.
67. Letouze E, Martinelli C, Loriot C, Burnichon N, Abermil N, Ottolenghi C, Janin M, Menara M, Nguyen AT, Benit P, et al. SDH mutations establish a hypermethylator phenotype in paraganglioma. *Cancer Cell* 2013, 23:739–752.
68. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF- α prolyl hydroxylase. *Cancer Cell* 2005, 7:77–85.
69. Yankovskaya V, Horsefield R, Tornroth S, Luna-Chavez C, Miyoshi H, Leger C, Byrne B, Cecchini G, Iwata S. Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science* 2003, 299:700–704.
70. Cardaci S, Zheng L, MacKay G, van den Broek NJ, MacKenzie ED, Nixon C, Stevenson D, Tumanov S, Bulusu V, Kamphorst JJ, et al. Pyruvate carboxylation enables growth of SDH-deficient cells by supporting aspartate biosynthesis. *Nat Cell Biol* 2015, 17:1317–1326.
71. Lussey-Lepoutre C, Hollinshead KE, Ludwig C, Menara M, Morin A, Castro-Vega LJ, Parker SJ, Janin M, Martinelli C, Ottolenghi C, et al. Loss of succinate dehydrogenase activity results in dependency on pyruvate carboxylation for cellular anabolism. *Nat Commun* 2015, 6:8784.
72. Guzy RD, Sharma B, Bell E, Chandel NS, Schumacker PT. 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* 2008, 28:718–731.
73. Selak MA, Duran RV, Gottlieb E. Redox stress is not essential for the pseudo-hypoxic phenotype of succinate dehydrogenase deficient cells. *Biochim Biophys Acta* 2006, 1757:567–572.
74. Wei MH, Toure O, Glenn GM, Pithukpakorn M, Neckers L, Stolle C, Choyke P, Grubb R, Middleton L, Turner ML, et al. Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer. *J Med Genet* 2006, 43:18–27.
75. Stein I, Peleg Y, Even-Ram S, Pines O. The single translation product of the FUM1 gene (fumarase) is processed in mitochondria before being distributed between the cytosol and mitochondria in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1994, 14:4770–4778.
76. Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung YL, Merino M, Trepel J, Zbar B, Toro J,

- 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* 2005, 8:143–153.
77. Xiao M, Yang H, Xu W, Ma S, Lin H, Zhu H, Liu L, Liu Y, Yang C, Xu Y, 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* 2012, 26:1326–1338.
78. Adam J, Hatipoglu E, O'Flaherty L, Ternette N, Sahgal N, Lockstone H, Baban D, Nye E, Stamp GW, Wolhuter K, 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* 2011, 20:524–537.
79. Ooi A, Wong JC, Petillo D, Roossien D, Perrier-Trudova V, Whitten D, Min BW, Tan MH, Zhang Z, Yang XJ, et al. An antioxidant response phenotype shared between hereditary and sporadic type 2 papillary renal cell carcinoma. *Cancer Cell* 2011, 20:511–523.
80. Adam J, Yang M, Bauerschmidt C, Kitagawa M, O'Flaherty L, Maheswaran P, Ozkan G, Sahgal N, Baban D, Kato K et al. A role for cytosolic fumarate hydratase in urea cycle metabolism and renal neoplasia. *Cell Rep* 2013, 3:1440–1448.
81. Zheng L, Mackenzie ED, Karim SA, Hedley A, Blyth K, Kalna G, Watson DG, Szlosarek P, Frezza C, Gottlieb E. Reversed argininosuccinate lyase activity in fumarate hydratase-deficient cancer cells. *Cancer Metab* 2013, 1:12.
82. Ternette N, Yang M, Laroyia M, Kitagawa M, O'Flaherty L, Wolhuter K, Igarashi K, Saito K, Kato K, Fischer R, et al. Inhibition of mitochondrial aconitase by succination in fumarate hydratase deficiency. *Cell Rep* 2013, 3:689–700.
83. Zheng L, Cardaci S, Jerby L, MacKenzie ED, Sciacovelli M, Johnson TI, Gaude E, King A, Leach JD, Edrada-Ebel R, et al. Fumarate induces redox-dependent senescence by modifying glutathione metabolism. *Nat Commun* 2015, 6:6001.
84. Bardella C, El-Bahrawy M, Frizzell N, Adam J, Ternette N, Hatipoglu E, Howarth K, O'Flaherty L, Roberts I, Turner G, et al. Aberrant succination of proteins in fumarate hydratase-deficient mice and HLRCC patients is a robust biomarker of mutation status. *J Pathol* 2011, 225:4–11.
85. Frezza C, Zheng L, Folger O, Rajagopalan KN, MacKenzie ED, Jerby L, Micaroni M, Chaneton B, Adam J, Hedley A, et al. Haem oxygenase is synthetically lethal with the tumour suppressor fumarate hydratase. *Nature* 2011, 477:225–228.
86. Galliani G, Monti D, Speranza G, Manitto P. Biliverdin as an electron transfer catalyst for superoxide ion in aqueous medium. *Experientia* 1985, 41:1559–1560.
87. Robertson P Jr, Fridovich I. A reaction of the superoxide radical with tetrapyrroles. *Arch Biochem Biophys* 1982, 213:353–357.
88. Kloosterhof NK, Bralten LB, Dubbink HJ, French PJ, van den Bent MJ. Isocitrate dehydrogenase-1 mutations: a fundamentally new understanding of diffuse glioma? *Lancet Oncol* 2011, 12:83–91.
89. Yan H, Bigner DD, Velculescu V, Parsons DW. Mutant metabolic enzymes are at the origin of gliomas. *Cancer Res* 2009, 69:9157–9159.
90. Cairns RA, Iqbal J, Lemonnier F, Kucuk C, de Leval L, Jais JP, Parrens M, Martin A, Xerri L, Brousset P, et al. IDH2 mutations are frequent in angioimmunoblastic T-cell lymphoma. *Blood* 2012, 119:1901–1903.
91. Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, Schenkein DP, Hezel AF, Ancukiewicz M, Liebman HM, et al. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. *Oncologist* 2012, 17:72–79.
92. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, et al. The consensus coding sequences of human breast and colorectal cancers. *Science* 2006, 314:268–274.
93. Kang MR, Kim MS, Oh JE, Kim YR, Song SY, Seo SI, Lee JY, Yoo NJ, Lee SH. Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. *Int J Cancer* 2009, 125:353–355.
94. Liu X, Kato Y, Kaneko MK, Sugawara M, Ogasawara S, Tsujimoto Y, Naganuma Y, Yamakawa M, Tsuchiya T, Takagi M. Isocitrate dehydrogenase 2 mutation is a frequent event in osteosarcoma detected by a multi-specific monoclonal antibody MsMab-1. *Cancer Med* 2013, 2:803–814.
95. Ang D, VanSandt AM, Beadling C, Warrick A, West RB, Corless CL, Troxell ML. Biphasic papillary and lobular breast carcinoma with PIK3CA and IDH1 mutations. *Diagn Mol Pathol* 2012, 21:221–224.
96. Zhang Y, Wei H, Tang K, Lin D, Zhang C, Mi Y, Wang L, Wang C, Wang M, Wang J. Mutation analysis of isocitrate dehydrogenase in acute lymphoblastic leukemia. *Genet Test Mol Biomarkers* 2012, 16:991–995.
97. Gaal J, Burnichon N, Korpershoek E, Roncelin I, Bertherat J, Plouin PF, de Krijger RR, Gimenez-Roqueplo AP, Dinjens WN. Isocitrate dehydrogenase mutations are rare in pheochromocytomas and paragangliomas. *J Clin Endocrinol Metab* 2010, 95:1274–1278.
98. Gravendeel LA, Kloosterhof NK, Bralten LB, van Marion R, Dubbink HJ, Dinjens W, Bleeker FE, Hoogenraad CC, Michiels E, Kros JM, et al. Segregation of non-p.R132H mutations in IDH1 in distinct molecular subtypes of glioma. *Hum Mutat* 2010, 31:E1186–E1199.

99. Xu X, Zhao J, Xu Z, Peng B, Huang Q, Arnold E, Ding J. Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity. *J Biol Chem* 2004, 279:33946–33957.
100. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 2009, 462:739–744.
101. Rendina AR, Pietrak B, Smallwood A, Zhao H, Qi H, Quinn C, Adams ND, Concha N, Duraiswami C, Thrall SH, et al. Mutant IDH1 enhances the production of 2-hydroxyglutarate due to its kinetic mechanism. *Biochemistry* 2013, 52:4563–4577.
102. Struys EA. D-2-Hydroxyglutaric aciduria: unravelling the biochemical pathway and the genetic defect. *J Inherit Metab Dis* 2006, 29:21–29.
103. Ward PS, Lu C, Cross JR, Abdel-Wahab O, Levine RL, Schwartz GK, Thompson CB. The potential for isocitrate dehydrogenase mutations to produce 2-hydroxyglutarate depends on allele specificity and subcellular compartmentalization. *J Biol Chem* 2013, 288:3804–3815.
104. Jin G, Reitman ZJ, Duncan CG, Spasojevic I, Gooden DM, Rasheed BA, Yang R, Lopez GY, He Y, McLendon RE, et al. Disruption of wild-type IDH1 suppresses D-2-hydroxyglutarate production in IDH1-mutated gliomas. *Cancer Res* 2013, 73:496–501.
105. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanthakumar A, Fernandez HF, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* 2010, 18:553–567.
106. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AW, Lu C, Ward PS, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 2012, 483:479–483.
107. Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagg EA, Rose NR, Leung IK, Li XS, Woon EC, Yang M, et al. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep* 2011, 12:463–469.
108. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α -ketoglutarate-dependent dioxygenases. *Cancer Cell* 2011, 19:17–30.
109. Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S, Losman JA, Joensuu P, Bergmann U, Gross S, et al. Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. *Nature* 2012, 483:484–488.
110. Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, Yu W, Li Z, Gong L, Peng Y, et al. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1 α . *Science* 2009, 324:261–265.
111. Losman JA, Looper RE, Koivunen P, Lee S, Schneider RK, McMahon C, Cowley GS, Root DE, Ebert BL, Kaelin WG Jr. (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science* 2013, 339:1621–1625.
112. Tarhonskaya H, Rydzik AM, Leung IK, Loik ND, Chan MC, Kawamura A, McCullagh JS, Claridge TD, Flashman E, Schofield CJ. Non-enzymatic chemistry enables 2-hydroxyglutarate-mediated activation of 2-oxoglutarate oxygenases. *Nat Commun* 2014, 5:3423.
113. Hartmann C, Meyer J, Bals J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W, et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. *Acta Neuropathol* 2009, 118:469–474.
114. Evans SM, Judy KD, Dunphy I, Jenkins WT, Hwang WT, Nelson PT, Lustig RA, Jenkins K, Magarelli DP, Hahn SM, et al. Hypoxia is important in the biology and aggression of human glial brain tumors. *Clin Cancer Res* 2004, 10:8177–8184.
115. Collingridge DR, Piepmeyer JM, Rockwell S, Knisely JP. Polarographic measurements of oxygen tension in human glioma and surrounding peritumoural brain tissue. *Radiother Oncol* 1999, 53:127–131.
116. Sondergaard KL, Hilton DA, Penney M, Ollerenshaw M, Demaine AG. Expression of hypoxia-inducible factor 1 α in tumours of patients with glioblastoma. *Neuropathol Appl Neurobiol* 2002, 28:210–217.
117. Grassian AR, Parker SJ, Davidson SM, Divakaruni AS, Green CR, Zhang X, Slocum KL, Pu M, Lin F, Vickers C, et al. IDH1 mutations alter citric acid cycle metabolism and increase dependence on oxidative mitochondrial metabolism. *Cancer Res* 2014, 74:3317–3331.