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Genetically-defined metabolic reprogramming in cancer

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Abstract

Oncogenes and tumor suppressors regulate cell metabolism. Evidence demonstrates that tumorigenic mutations in these genes tend to orchestrate metabolic activity into a platform that promotes cell survival, growth and proliferation. Recent work has shown that some metabolic enzymes are also mutated in cancer, and that these mutations may influence malignancy directly. Thus, these enzymes seem to function as oncogenes and tumor suppressors, and would appear to be compelling targets for therapy. Here, we review several enzymes mutated in cancer – phosphoglycerate dehydrogenase, isocitrate dehydrogenases 1 and 2, succinate dehydrogenase, and fumarate hydratase – and discuss exciting new work that has begun to pull back the curtain on how mutations in these enzymes influence tumorigenesis.

Genomic alteration of metabolic enzymes in cancer

Cancer is a genetic disease caused primarily by mutations in oncogenes and tumor suppressors. These two classes of genes normally regulate tissue homeostasis, and their perturbed function in cancer causes unwarranted cell survival and growth, culminating in tumorigenesis. Tumors often display oncogene-activating genomic alterations such as gene amplification and gain-of-function point mutations, and/or tumor-suppressor inactivating mutations such as gene deletion, loss-of-function point mutations, or epigenetic silencing. Together, these mutations enable cells to acquire the stereotypical capabilities (“hallmarks”) of malignancy [1]. Over the past decade, a large body of work has demonstrated that most if not all oncogenes and tumor suppressors also regulate metabolism, so that mutations in these genes orchestrate nutrient utilization in a manner that facilitates cell survival and growth. Common metabolic effects of tumorigenic mutations include activation of aerobic glycolysis (the “Warburg effect”, see Box 1 and Glossary), glutaminolysis, and anaplerosis, which collaborate to produce energy and macromolecular precursors needed to produce lipids, nucleic acids, and proteins for dividing cells (Box 1) [2, 3]. In experimental systems, blockade of these metabolic activities has been demonstrated to suppress tumor cell growth in culture and *in vivo*. Thus, deregulated cell metabolism is now considered a hallmark of

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malignancy and an essential effect of tumorigenic mutations [4]. The metabolic effects of oncogenes and tumor suppressors have been extensively reviewed elsewhere [5, 6].

Text Box 1

Major oncogene-regulated metabolic pathways in cancer

Starting in the 1920s, Otto Warburg demonstrated high rates of glucose consumption and lactate secretion in tumor tissue, even when the tissue had access to abundant oxygen [67]. Aerobic glycolysis, or the “Warburg effect,” is the most frequently cited metabolic feature of cancer cells, and is stimulated by a large number of oncogenes. Other metabolic pathways stimulated by oncogenes and cited as components of tumor metabolism include glutamine catabolism (glutaminolysis); *de novo* synthesis of fatty acids and lipids; and anaplerosis, pathways that enable the TCA cycle to provide biosynthetic precursors. These pathways tend to occur concurrently in cancer cells, providing a platform that supports rapid cell growth and proliferation [68].

Remarkably, in some situations, metabolic enzymes independently function as oncogenes or tumor suppressors, suggesting that primary metabolic disturbances can drive cells towards a malignant phenotype, or at least facilitate tumorigenesis [7]. Genes encoding these enzymes are mutated in the genome just like traditional tumor suppressors and oncogenes. Tumors harboring these metabolic mutations have generated a tremendous amount of interest over the past few years. They are both interesting and important, because cells derived from these tumors display metabolic activities that differ significantly, in some cases profoundly, from that of normal tissue. Understanding these perturbed metabolic networks may shed light on the biological basis of malignancy, and may enable the development of therapies based on selective targeting of tumor metabolism [8]. Here, we review several metabolic enzymes that are mutated in human cancer, and discuss efforts to understand why these mutations appear to facilitate tumorigenesis.

Phosphoglycerate Dehydrogenase (PHGDH) – An enzyme commonly amplified in human tumors

Serine and glycine are nonessential amino acids that serve as intermediates in numerous metabolic pathways (Figure 1). Both are relatively abundant in human plasma, typically exceeding 100 μmol/L, and many cancer cells can avidly import them from the culture medium. Nevertheless, a significant fraction of human cancer cells appear to rely on *de novo* synthesis of these two amino acids [9, 10]. This pathway begins with the oxidation of a glycolytic intermediate, 3-phosphoglycerate (3PG), to 3-phospho-hydroxypyruvate (3POHPyr) by the enzyme phosphoglycerate dehydrogenase (PHGDH). Transamination of 3POHPyr by phosphoserine aminotransferase-1 (PSAT1) generates phosphoserine, which is then converted to serine. Serine may donate a methyl group to the cellular one-carbon pool, and in doing so is converted to glycine. Together, both serine and glycine feed a large number of metabolic pathways that support cell survival and growth (Figure 1). Genomic amplification of the *PHGDH* gene on chromosome 1p12 occurs in some 16% of human cancers, including 40% of melanomas and 6% of breast cancers, and an even larger fraction of tumors display enhanced expression of this enzyme, regardless of copy number gain [9, 10]. Silencing PHGDH in human tumor cell lines with high levels of expression limits their growth in culture and in mouse models of cancer, identifying PHGDH as a potential therapeutic target in tumors leveraged towards *de novo* serine biosynthesis.

An accelerated flux through the serine biosynthetic pathway may provide a number of advantages for growing cancer cells, perhaps contributing to oncogenesis. First, serine is

required as a precursor for the biosynthesis of lipids, proteins, nucleotides and amino acids [11, 12]. Thus, the ability to replenish the serine and glycine pools may allow cells to survive the stress associated with the aberrant activation of oncogenes, which in some contexts may reduce nucleotide availability [13]. Oncogenes also induce production of reactive oxygen species (ROS), which stimulate cellular senescence, unless cells are armed with robust antioxidant defense systems [14]. Cells may capitalize on enhanced serine/glycine biosynthesis to help mitigate this stress, because glycine is a precursor for the major antioxidant glutathione [15].

Interestingly, while silencing PHGDH may suppress cancer cell growth, it does not necessarily do so simply by limiting intracellular serine/glycine. Possemato et al. found that cells with high PHGDH activity also use this pathway to produce up to 50% of their α -ketoglutarate supply [10]. α -ketoglutarate is produced by PSAT1 as it transfers an amino group from glutamine derived-glutamate to 3POHPyr. α -ketoglutarate is a crucial intermediate in cancer cells, because it can be metabolized in the mitochondria to generate ATP, and to feed pools of TCA cycle intermediates, many of which serve as macromolecular precursors [16]. Cancer cells, at least in culture, express a number of other highly-active transaminases, including alanine aminotransferase (glutamic-pyruvic transaminase) and aspartate aminotransferase (glutamic-oxaloacetic transaminase). These other transaminases, rather than PSAT1, often account for the majority of glutamine-derived α -ketoglutarate in human cancer cells [17]. Thus, it will be interesting to determine why a subset of cancer cells seem to use PSAT1 as the preferred source of α -ketoglutarate, or whether serine biosynthesis provides other metabolic advantages yet to be uncovered in these cells.

Beyond orchestrating a growth-promoting metabolic phenotype, evidence also suggests that PHGDH, when expressed at high levels, may have properties that prime cells for transformation. Over-expression of catalytically active PHGDH, but not a hypomorphic mutant, in breast epithelial cells induces luminal filling, abnormal nuclear morphology, anchorage-independence and disturbed cell polarity [9]. All of these changes are associated with cellular transformation, suggesting that PHGDH over-expression enhances the acquisition of malignant properties.

Isocitrate dehydrogenase 1 and 2: Point mutations create a neomorphic enzyme activity and production of an “oncometabolite”

Isocitrate dehydrogenases (IDH) serve important roles in normal cell metabolism and growth (Figure 2) [18]. These enzymes catalyze the oxidative decarboxylation of isocitrate to α -ketoglutarate and are distinguishable from one another based on sub-cellular localization and cofactor preference. IDH3 is a multi-subunit enzyme that is localized within the mitochondrial matrix, and uses NAD⁺ as a cofactor in an irreversible reaction that generates α -ketoglutarate in the TCA cycle. IDH1 and IDH2, which share no sequence similarity with IDH3, use NADP⁺ as a cofactor in reversible reactions that occur in the cytoplasm and mitochondria, respectively [19].

In 2008, genome-sequencing efforts in glioblastoma multiforme (GBM) led to the surprising observation that a subset of these aggressive brain tumors contains mutations in *IDH1* [20]. IDH1 mutations were preferentially observed in young patients with secondary GBM, i.e. with aggressive gliomas that had arisen from lower-grade, slowly-growing tumors. A follow-up study examining more than 400 central nervous system tumors determined that some 70% of secondary GBM or World Health Organization Grade II and III astrocytomas and oligodendrogiomas contained *IDH1* mutations, and other tumors from these groups had mutations in *IDH2* [21]. In both studies, the median survival of patients with *IDH*-mutant

tumors was more than double that of patients lacking these mutations. Subsequently, *IDH1* and *IDH2* mutations were also found in other cancers, including acute myeloid leukemia (AML) and chondrosarcoma [21–24].

Although these *IDH1* and *IDH2* mutations inactivate the canonical, NADPH-producing activity of these enzymes [21], several pieces of evidence argued against the concept that *IDH1* and *IDH2* are tumor suppressors. First, in human gliomas, all mutations were somatically acquired. Second, the tumor-associated mutations were restricted to the isocitrate binding site, and overtly inactivating mutations like frame shifts or stop codons were not observed. Third, only one *IDH1* or *IDH2* mutation was identified in each tumor, with retention of one wild-type allele and no loss of heterozygosity. All of these observations argue against a simple loss-of-function model, and suggest that the mutant *IDH* alleles had acquired a new activity that promotes tumor formation. Indeed, a metabolomic analysis of human GBM cells engineered to over-express mutant *IDH1*, identified large amounts of *R*(−)-2-hydroxyglutarate ((*R*)-2HG), the reduced form of α-ketoglutarate [25] (Figure 2). This metabolite was also abundant in human tumors with mutant *IDH1*, but not wild-type *IDH1*, and *in vitro* studies revealed that the mutant allele possesses a neomorphic enzyme activity that enables it to convert α-ketoglutarate directly to (*R*)-2HG in the presence of NADPH [25]. Mutations in *IDH2* also produce (*R*)-2HG from α-ketoglutarate and result in 2HG accumulation in primary human AML samples [26].

2HG is a compelling metabolite in human disease. Both of its enantiomers, (*R*)-2HG and (*S*)-2HG (also known as D-2HG and L-2HG) were previously observed to accumulate in rare, autosomal recessive metabolic disorders caused by loss of the dehydrogenases that convert these metabolites to α-ketoglutarate, D-2HG-dehydrogenase (D2HGDH) and L-2HG-dehydrogenase (L2HGDH) [27]. Children with homozygous mutations in L2HGDH accumulate (*S*)-2HG, but not (*R*)-2HG, in all body fluids, starting in childhood or earlier. The main clinical features of L2HGDH deficiency are developmental delay, seizures, ataxia, and abnormalities of the white matter and basal ganglia. Several of these children have also developed tumors, particularly in the brain, raising the possibility that (*S*)-2HG contributes to oncogenesis [27]. Children with homozygous mutations in D2HGDH accumulate (*R*)-2HG, but not (*S*)-2HG, starting in early in life, and these patients develop a syndrome of developmental delay, hypotonia, and seizures. Germline, monoallelic gain-of-function *IDH2* mutations, similar to those observed in gliomas, were recently identified in a subset of children systemic elevations of (*R*)-2HG [28]. These children display phenotypic overlap with D2HGDH deficiency, and approximately half of them also develop cardiomyopathy [27]. Interestingly, despite the fact that (*R*)-2HG accumulates in tumors with monoallelic, somatically-acquired *IDH1* and *IDH2* mutations, neither systemic D2HGDH deficiency nor germline *IDH2* mutations have so far been associated with cancer [29]. This seems to suggest that (*R*)-2HG is a context-specific “oncometabolite,” perhaps requiring exposure to susceptible cell types and/or a permissive developmental state to elicit malignancy.

The specific mechanisms for (*R*)-2HG’s putative transforming properties are now the subject of intense study. Due to its close structural similarity to α-ketoglutarate, (*R*)-2HG modulates the activity of many enzymes that use α-ketoglutarate as a substrate. These include the α-ketoglutarate-dependent dioxygenases, which comprise a large family of enzymes that carry out diverse functions such as prolyl hydroxylation, histone demethylation and epigenetic modification of DNA (Figure 2) [30, 31]. In gliomas, (*R*)-2HG produced by mutant *IDH1* substantially and stably alters the DNA methylome and is sufficient to establish the CpG island methylator phenotype, which defines a clinically distinct subset of tumors [32]. Enhanced DNA methylation and epigenetic remodeling is also common in AML, where it frequently occurs through deletion of the 5-methylcytosine hydroxylase *TET2*, another α-ketoglutarate-dependent enzyme [33]. AML samples with

IDH1/2 mutations exhibit a similar hypermethylation phenotype, yet these mutations are mutually exclusive with *TET2* mutations [34]. Transient transfection of mutant *IDH1/2* was sufficient to inhibit *TET2* function in cultured cells, suggesting that (*R*)-2HG-producing *IDH1/2* mutations are functionally redundant with inactivating *TET2* mutations [31]. Expression of these mutations also impairs differentiation of primary mouse bone marrow cells [34] and suppresses histone demethylation and adipocyte differentiation in culture [35]. Together, these observations suggest that (*R*)-2HG-producing *IDH1/2* mutations promote malignancy by remodeling the epigenetic landscape in a way that arrests cellular differentiation.

(*R*)-2HG also modulates the activity of the hypoxia-inducible transcription factors (HIFs) by altering the stability of their α -subunits. These subunits (HIF-1 α and HIF-2 α) are normally targeted for degradation in the presence of oxygen, through post-translational modifications, particularly by the EGLN family of prolyl hydroxylases. At low millimolar levels similar to those found in *IDH1/2*-mutant gliomas, (*R*)-2HG selectively enhances prolyl hydroxylase activity *in vitro* by acting as a co-substrate for the enzyme [36]. This is associated with enhanced degradation of HIF- α subunits in cell lines expressing *IDH1* mutations, and with blunted expression of HIF target genes in *IDH1/2*-mutant gliomas [36]. Because EGLN expression was required for maximal proliferation of *IDH1*-mutant cells in culture, the findings suggest that (*R*)-2HG supports a program of cell growth.

Work on *IDH1/2* mutations in cancer has revealed a system in which a primary metabolic disturbance elicits non-metabolic effects that promote malignancy. It is unclear whether suppressing (*R*)-2HG formation in tumors will reverse these effects. However, the relatively favorable prognosis in gliomas with *IDH1/2* mutations emphasizes the prognostic utility of detecting (*R*)-2HG in tumors. Indeed, methods to image (*R*)-2HG non-invasively in human cancer patients by magnetic resonance spectroscopy have already been reported, and will likely disseminate rapidly into routine clinical practice [37–40].

Succinate Dehydrogenase and Fumarate Hydratase: TCA cycle enzymes and tumor suppressors

Succinate dehydrogenase (SDH) and fumarate hydratase (FH) catalyze sequential steps within the TCA cycle and are mutated in human tumors (Figure 3A). SDH is a multi-subunit complex that also functions as Complex II of the electron transport chain (ETC). SDH oxidizes succinate in a reaction that generates fumarate and FADH₂, and donates electrons to the ETC [41]. Mutations in genes encoding SDH subunits were identified in familial forms of the neuroendocrine tumors pheochromocytoma (PCC) and paraganglioma (PGL) [42–45]. Affected individuals inherit a loss of function mutation in one allele of a gene encoding an SDH subunit, and their tumors display loss of expression of the other allele, through somatic deletion or other mechanisms. Thus, these genes follow the typical inheritance pattern of tumor suppressors. In some of these tumor types, penetrance depends on the parent from whom the germline mutation is inherited. For cases of PGL caused by mutations in *SDHD*, only paternally-acquired mutations are associated with tumors, suggesting that the maternally-inherited allele is imprinted [46]. Interestingly, while *SDHD* itself shows no direct evidence of methylation, boundary elements, including the alternative promoter of a large intergenic non-coding RNA (lincRNA), are heavily methylated on the maternally-inherited allele [47]. Furthermore, this region displays tissue-specific methylation, particularly in the adrenal glands, the anatomic site of PCC. This may provide an explanation both for the inheritance pattern and for the tumor type in this disease.

Fumarate hydratase is a TCA cycle enzyme that catalyzes the hydration of fumarate to malate. Biallelic germline FH mutations cause a rare metabolic disorder characterized by

severe encephalopathy and mental retardation [48]. In 2002, it was discovered that heterozygous mutations in FH underlie a familial syndrome of benign leiomyomas of the skin and uterus, and highly aggressive renal tumors, termed hereditary leiomyomatosis and renal cell cancer (HLRCC) [49]. As in the familial forms of PCC and PGL, individuals with HLRCC inherit one loss of function allele and somatically lose the other allele in the tumors. The inherited *FH* mutations severely reduce enzyme activity, causing the tumors to accumulate high levels of fumarate [50].

The overtly-dysfunctional TCA cycles of FH- and SDH-deficient tumors present significant metabolic challenges, because the TCA cycle is a central pathway for energy formation and for the production of macromolecular precursors. Several lines of investigation have revealed mechanisms by which cells cope with this problem. First, succinate and fumarate function in a feed-forward system to allow cells to up-regulate glycolytic activity, which allows them to produce ATP independently of the TCA cycle. Both fumarate and succinate inhibit α -ketoglutarate-dependent prolyl hydroxylases, resulting in the normoxic stabilization of HIF- α subunits and constitutive expression of HIF transcriptional targets, particularly glycolytic genes, in human cells [51, 52]. Second, the survival of FH-deficient murine kidney cells requires the induction of linked metabolic activities, heme biosynthesis and degradation, that remove some of the excess carbon from the dysfunctional TCA cycle and generate reducing equivalents in the form of NADH (Figure 3) [53]. Silencing one of the enzymes in this pathway, heme oxygenase-1 (HMOX1), using RNA interference or a chemical inhibitor, kills FH-deficient human and mouse kidney cells in culture, but is well tolerated by cells expressing wild-type FH [53].

Third, FH deficiency precludes tumor cells from generating several of the TCA cycle intermediates through conventional oxidative metabolism. These include malate, a source of NADPH for redox homeostasis; oxaloacetate, a precursor for nucleotide biosynthesis; and citrate, the primary source of acetyl-CoA for *de novo* fatty acid synthesis [15, 16]. To compensate for this, human FH-deficient renal tumor cells redirect part of the TCA cycle. Rather than using IDH as an oxidative decarboxylase to convert isocitrate to α -ketoglutarate, they carboxylate α -ketoglutarate to produce isocitrate. This reaction, called reductive carboxylation, uses NADPH-dependent IDH isoforms, and consumes glutamine-derived α -ketoglutarate. Subsequent metabolism of isocitrate generates citrate, which is then cleaved to produce oxaloacetate and acetyl-CoA. This acetyl-CoA pool is the major source of carbon for fatty acid synthesis, while the oxaloacetate is reduced to form malate, thus “back-filling” the pools of TCA cycle intermediates impaired by FH deficiency (Figure 3A). This pathway of glutamine-dependent reductive carboxylation appears to be a robust mechanism allowing cells to maintain growth during impaired oxidative metabolism, because it is also observed in human cancer cell lines with mutations in the ETC or in the Von-Hippel-Lindau (*VHL*) tumor suppressor, and in cells subjected to hypoxia, all of which negatively impact oxygen-dependent mitochondrial enzymes [54–57].

Finally, high levels of fumarate and succinate can induce aberrant patterns of gene expression. Succinate inhibits the histone demethylase JMJD3, leading to a global increase in steady state histone methylation [58]. Succinate inhibition of the EGLN3 prolyl hydroxylase leads to a suppression of neuronal apoptosis in PGL and PCC tumors [59, 60]. As a result of its electrophilic properties, fumarate modifies cysteine residues on cellular proteins, producing an S-(2-Succinyl)cysteine (2SC) adduct in a Michael addition reaction called succination [61]. Succination has been reported to impair protein function. For example, Kelch-like ECH-associated protein 1 (Keap1) is a major cellular electrophile sensor and negative regulator of the transcription factor Nuclear Factor E2-related factor 2 (Nrf2) (Figure 3B). In the absence of electrophiles, Keap1 targets Nrf2 for ubiquitination and degradation. In the presence of electrophiles, the Keap1-Nrf2 complex is disrupted, and

Nrf2 induces expression of a suite of genes involved in antioxidant defense. In cells with FH deficiency, fumarate-mediated succination of Keap1 elicits an Nrf2 response, maintaining constitutively high expression of Nrf2 targets [62–64]. Interestingly, one of these targets is *HMOX1*, which encodes the enzyme required for heme degradation in FH-deficient cells, suggesting that fumarate-dependent suppression of Keap1 may promote cell survival. It is unclear what role the Keap1/Nrf2 system plays in tumorigenesis generally, but several lines of evidence suggest that it promotes tumor formation and/or growth. Keap1 mutations are frequently observed in solid human tumors, leading to the proposal that it functions as a tumor suppressor [65]. Furthermore, ablation of the *NRF2* gene reduces tumor formation in an oncogenic *KRAS* mouse model [66]. Thus, fumarate-mediated suppression of Keap1 may contribute to tumor development in the setting of FH deficiency.

Concluding Remarks

In some forms of cancer, metabolic enzymes are altered by the same mutational mechanisms observed in classical oncogenes and tumor suppressors: genomic amplification, activating mutations, and loss-of-function mutations. All of these metabolic mutations reprogram cellular metabolism in ways that either prime cells for transformation or otherwise contribute to oncogenesis. In the case of *PHDGH*, a metabolic activity is enhanced via genomic amplification, supporting cell growth and perhaps stimulating the development of malignant properties. Active-site mutations in *IDH1/2* induce a neomorphic enzyme activity, eliciting epigenetic effects that would have been impossible to predict solely from the primary metabolic disturbance. SDH and FH function genetically as classical tumor suppressors despite the expectation that loss of these enzyme activities should severely impair cellular fitness. While nearly a century of research in cancer metabolism has focused on the enhancement of core metabolic pathways (glycolysis, glutaminolysis, lipid synthesis, etc.) in aggressive tumors, these recently-discovered metabolic outliers may provide unique opportunities to understand why particular metabolic changes lead to cancer in a tissue-specific manner (Box 2). In order to solve this problem, it will be necessary to understand how metabolic perturbations influence processes beyond bioenergetics, energy storage, and macromolecular synthesis, the traditional roles of metabolism, and support the hallmarks of malignancy. Tumors with primary metabolic disturbances should also hold significant potential in translational studies. Diagnostic imaging of abundant metabolites is already possible in gliomas containing (*R*)-2HG, and may be possible for fumarate and succinate as well. Importantly, the significant departures from normal metabolism described in these tumors may widen the therapeutic window for treatments designed to attack the mutant pathways, enabling selective toxicity to be achieved in the tumor cells.

Text Box 2

Outstanding questions

- Do mutations in other metabolic enzymes besides the ones discussed here occur in human cancer?
- Are mutations in other tumor suppressors and oncogenes, acting in concert with mutations in metabolic enzymes, required for malignant transformation? Are there any contexts in which metabolic mutations are sufficient for oncogenesis?
- Are there other extra-metabolic roles of (*R*)-2HG, succinate and fumarate that contribute to malignant transformation and tumor growth?
- What are the mechanisms that restrict tumor development to specific organs following enzyme mutations?

- How well does the abundant information on the metabolism of cultured cancer cells –acquired over many decades – correlate with *bona fide* metabolism of live tumors growing in their native microenvironment *in vivo*?
- Would reversion of tumor metabolism to “normal” pathways impair tumor growth, cause tumor regression, or stimulate other therapeutically useful effects?

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Glossary

Acute myeloid leukemia (AML)	a cancer of the myeloid line of blood cells and the most common acute leukemia in adults
Chondrosarcoma	a cartilage-forming cancer of the bone
Fumarate hydratase (FH)	TCA cycle enzyme that converts fumarate to malate. Mutations in FH are observed in families with HLRCC (see above). In individuals with this disorder, one mutated allele of <i>FH</i> is inherited, and expression of the other allele is lost in the tumor, demonstrating that <i>FH</i> functions as a tumor suppressor in this disease
Glioblastoma multiforme (GBM)	the most common and aggressive malignant primary brain tumor in humans
Hereditary leiomyomatosis and renal cell cancer (HLRCC)	a rare cancer syndrome characterized by benign smooth muscle tumors and aggressive collecting duct or Type 2 papillary renal cell carcinoma
Isocitrate dehydrogenases (IDH)	These enzymes catalyze the conversion of isocitrate to α -ketoglutarate. Low-grade gliomas, acute myelogenous leukemias, chondrosarcomas and other human tumors contain somatically-acquired mutations in either of two IDH isoforms, <i>IDH1</i> or <i>IDH2</i> . <i>IDH1</i> and <i>IDH2</i> are considered to be oncogenes in these tumors. However, unlike most oncogenes, mutations in <i>IDH1</i> and <i>IDH2</i> do not simply accentuate their canonical enzyme activity; rather mutant forms of <i>IDH1</i> and <i>IDH2</i> acquire the ability to produce an oncometabolite, <i>R</i> (–)-2-hydroxyglutarate (see below), from α -ketoglutarate
Phosphoglycerate dehydrogenase (PHGDH)	An enzyme involved in the <i>de novo</i> synthesis of serine from glucose. It converts a glycolytic intermediate, 3-phosphoglycerate, to 3-phospho-hydroxypyruvate. PHGDH protein is highly expressed in a substantial fraction of human cancers, and amplification of the <i>PHGDH</i> gene on chromosome 1p12 accounts for many of these. PHGDH is considered a therapeutic target in cancer, because some tumor cells

Pheochromocytoma (PCC)	displaying high flux through the pathway of <i>de novo</i> serine biosynthesis require PHGDH for growth in culture and <i>in vivo</i> a rare neuroendocrine tumor of the adrenal gland, often secreting excessive amounts of catecholamines
Paraganglioma (PGL)	a rare, generally benign neuroendocrine tumor that may develop in the head, neck, thorax and abdomen
Reductive carboxylation	Enzymatic reaction in which α -ketoglutarate, usually generated from glutamine catabolism, is carboxylated in the presence of CO ₂ and NADPH, to produce isocitrate. This reaction enables cells to maintain production of TCA cycle metabolites even when function of the cycle is severely impaired by FH deficiency or other processes
R(-)-2-hydroxyglutarate ((R)-2HG)	An “oncometabolite” produced via the neomorphic enzyme activity of mutant <i>IDH1</i> and <i>IDH2</i> . The metabolite results from the NADPH-dependent reduction of α -ketoglutarate. Tumors with <i>IDH1</i> or <i>IDH2</i> mutations may accumulate (R)-2HG to levels of several millimoles/L. (R)-2HG alters the function of a large number of enzymes that use α -ketoglutarate as a substrate, and these effects are implicated in the oncogenic potential of mutant <i>IDH1</i> and <i>IDH2</i>
Succinate dehydrogenase (SDH)	Tricarboxylic acid (TCA) cycle enzyme that converts succinate to fumarate, and also functions as complex II of the electron transport chain. SDH consists of four structural subunits, all encoded by genes in the nuclear genome. Mutations in any of these genes, or in a gene encoding an assembly factor for the complex, can be observed in patients with paraganglioma and pheochromocytoma. In individuals with familial forms of these diseases, the inheritance pattern demonstrates that SDH is a tumor suppressor
Warburg effect	a metabolic phenomenon characterized by high rates of glucose consumption and lactate secretion in the presence of oxygen. Named for the German biochemist Otto Warburg

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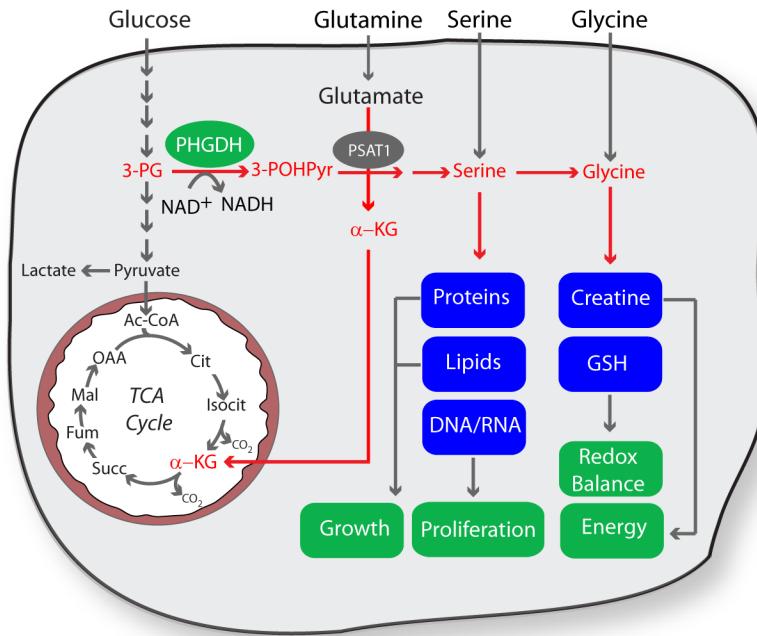
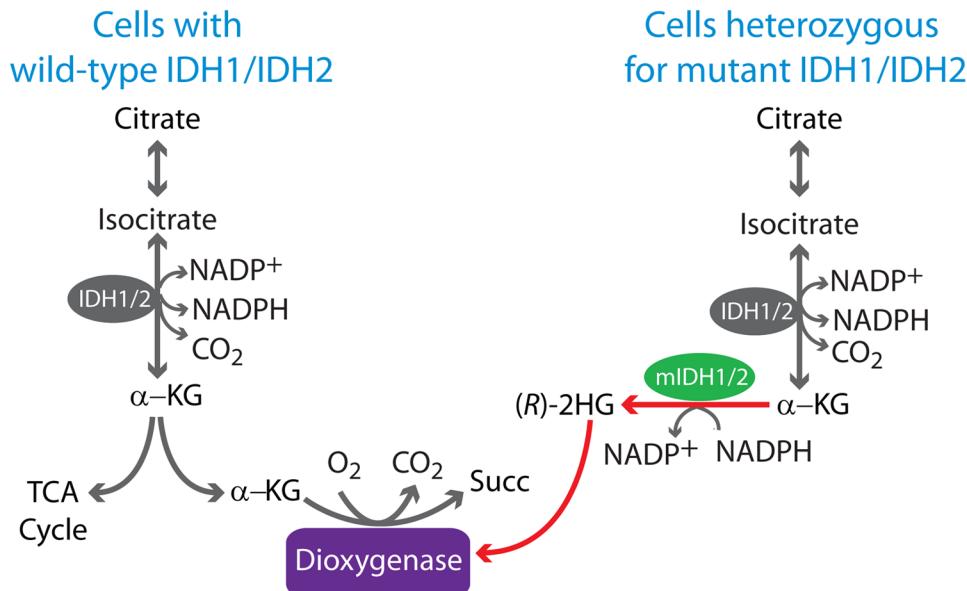


Figure 1. Phosphoglycerate dehydrogenase is over-expressed in some cancers and catalyzes a growth-promoting metabolic pathway

Glycolytic cancer cells convert glucose into pyruvate, which can then be oxidized in the mitochondria or converted into lactate. Cells containing enhanced expression of the enzyme phosphoglycerate dehydrogenase (PHGDH), either as the result of genomic amplification of its gene on chromosome 1p12 or through other mechanisms, divert 3-phosphoglycerate (3-PG) away from glycolysis into the serine/glycine biosynthetic pathway (red arrows), which generates a number of important metabolic intermediates. Along this pathway, transamination of 3-phospho-hydroxypyruvate (3-POHPyr) by the enzyme phosphoserine aminotransferase-1 (PSAT1) generates α-ketoglutarate (α-KG), which can then be oxidized in the tricarboxylic acid cycle (TCA). Serine and glycine are used to produce glutathione, proteins, nucleic acids, phospholipids and sphingolipids, and other molecules required for cell growth and proliferation. Abbreviations: Ac-coA, acetyl-coA; Cit, citrate; Isocit, isocitrate; Succ, succinate; Fum, fumarate; Mal, malate; OAA, oxaloacetate; NAD, nicotine adenine dinucleotide.



αKG-dependent dioxygenases Effect of (R)-2HG (references)

Prolyl Hydroxylases	Stimulation; increased HIF α degradation [36]
JMJC Histone Demethylases	Inhibition; enhanced histone methylation [30, 35]
TET2 5-mC Hydroxylase	Inhibition; decreased 5-mC in DNA [31, 34]

Figure 2. Mutant IDH1/2 enzymes produce an oncometabolite with pleiotropic effects on cell signaling and epigenetics

Normal cells contain wild type *IDH1* and *IDH2* (gray). These enzymes catalyze the reversible conversion of isocitrate to α-ketoglutarate (α-KG), generating NADPH and CO₂. α-KG can be oxidized in the TCA or used as a cofactor by α-KG-dependent dioxygenase enzymes. Tumor cells with somatically-acquired, heterozygous active site mutations in *IDH1* or *IDH2* (mIDH1/2, green) display a neomorphic enzyme activity that reduces α-KG to *R*(-)2-hydroxyglutarate ((R)-2HG), using NADPH as a cofactor. Due to its structural similarity to α-KG, (R)-2HG modulates the function of α-KG-dependent dioxygenases, stimulating prolyl hydroxylase activity and inhibiting a number of enzymes that regulate histone and DNA modifications. Together, these processes exert complex effects on gene expression that likely contribute to the malignancy of *IDH1/2*-mutant cells.

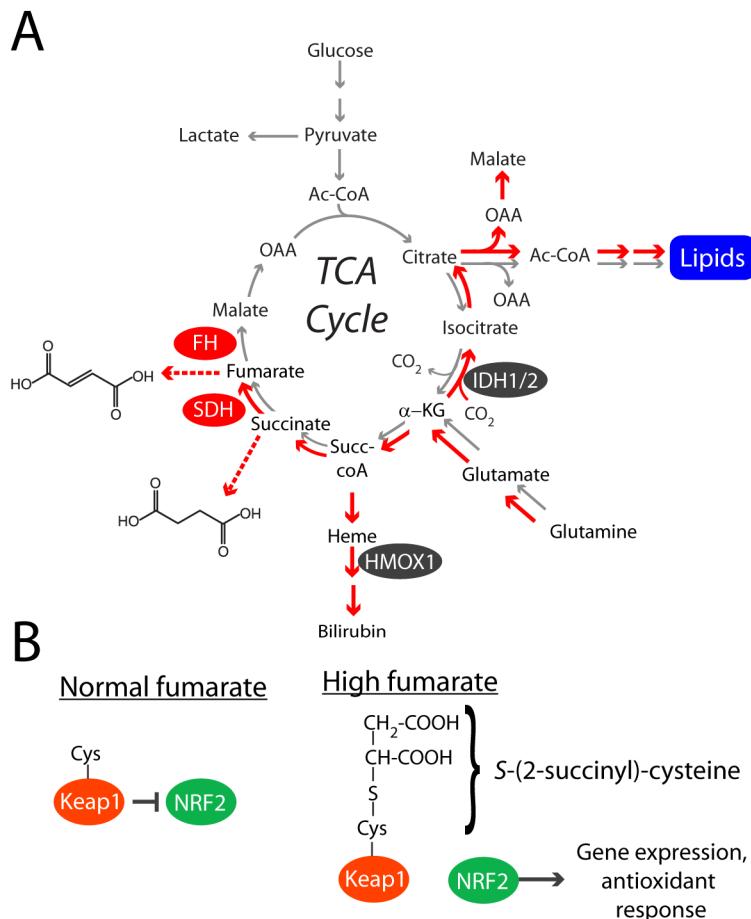


Figure 3. Effects of mutation of TCA cycle enzymes on metabolism and gene expression

(A) Succinate dehydrogenase (SDH) and fumarate hydratase (FH) are TCA cycle enzymes and tumor suppressors. In normal cells, succinate and fumarate are generated through oxidative metabolism of glutamine-derived α-KG (gray arrows). Subsequent metabolism around the TCA cycle generates citrate for lipid synthesis. SDH and FH deficiency interrupt this pathway, with accumulation of succinate and fumarate, respectively. FH-deficient cells redirect TCA cycle metabolism in two ways (red arrows). First, the cells shunt succinyl-CoA into a pathway of heme biosynthesis and degradation, culminating in the secretion of bilirubin. Inhibiting Heme Oxygenase-1 (HMOX1) in this pathway selectively kills cells with FH deficiency. Second, in order to produce citrate, the cells use reductive carboxylation of glutamine-derived α-KG. IDH1 and/or IDH2 participate in this reaction, and subsequent metabolism of citrate produces acetyl-CoA for fatty acid/lipid synthesis, and other TCA cycle intermediates like oxaloacetate and malate, which are normally produced downstream of FH. (B) Keap1 is an electrophile sensor. In the absence of fumarate and other electrophiles, Keap1 negatively regulates the transcription factor Nrf2, targeting it for degradation. In FH-deficient cells, cysteine residues on Keap1 are modified by fumarate-dependent succination, in which cysteine is converted to S-(2-succinyl)-cysteine. Nrf2, now active, can activate the transcription of genes involved in the antioxidant response.

Abbreviations: Ac-CoA, acetyl-CoA; Succ-CoA, succinyl-CoA; OAA, oxaloacetate; HMOX1, heme oxygenase-1; IDH1/2, isocitrate dehydrogenase isoforms 1 and 2; Cys, cysteine.