SCIENCE CHINA
Life Sciences

• COVER ARTICLE •
• CUSBEA ARTICLE SERIES • REVIEW •

December 2013 Vol.56 No.12: 1067–1075
doi: 10.1007/s11427-013-4549-2

Metabolic alteration in tumorigenesis

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Received July 16, 2013; accepted August 7, 2013; published online October 9, 2013

Altered metabolism in cancer was first discovered by Otto Warburg early last century. Although the Warburg Effect has been widely used in tumor detection, relatively little progress had been made in mechanistic understanding of cancer metabolism in the subsequent eight decades. Genetic studies have recently identified mutations in human cancer targeting multiple enzymes involved in intermediate metabolism. One emerging mechanism common to these mutant enzymes is the accumulation of a metabolite that alters the epigenetic control.

metabolite, epigenetic, tumorigenesis

Citation: Yang H, Xiong Y, Guan K L. Metabolic alteration in tumorigenesis. Sci China Life Sci, 2013, 56: 1067−1075, doi: 10.1007/s11427-013-4549-2

Otto Warburg [1,2] first discovered that cancer cells displayed enhanced glucose uptake and aerobic glycolysis, a phenomenon often referred as the Warburg Effect nowadays. Although the mechanism underlying the Warburg Effect is still not fully understood, increased uptake of glucose provides the basis to exploit its clinical application by ^{18}F-deoxyglucose positron emission tomography (PET) for tumor detection [3,4]. Decades after the Warburg Effect was discovered, the mechanistic insights of how metabolic alterations contribute to tumorigenesis are just emerging. Recent studies have revealed that eight metabolic genes, *FH*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *IDH1* and *IDH2*, encoding for subunits of four different metabolic enzymes, fumarate hydratase (FH), succinate dehydrogenase (SDH), and isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2), were mutated in a number of human cancers [5]. These findings provide compelling genetic evidence supporting the notion that altered metabolism contributes to, as opposed to the consequence of, tumorigenesis. Here, we first briefly discuss how metabolism is reprogrammed to support cancer cell proliferation. We then focus on one emerging mechanism that is common to the mutations targeting all four metabolic enzymes in accumulating a metabolite to alter the epigenetic modifications in human cancer.

1 Cancer cells reprogram metabolism for proliferation

Cancer cells require much more energy, reductive power, and intermediates as precursors than normal cells for the biosynthesis of macromolecules to support the high rate of cell growth and proliferation. This was what Otto Warburg has observed more than 80 years ago as perhaps the first phenotype of tumor cells that they consume glucose at a surprisingly high rate compared with normal cells, and accumulate a significant amount of lactate rather than oxidiz-

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ing glucose completely to water and CO_2 [1,2,6,7]. In other words, the tumor cells appear to favor the anaerobic metabolism even in the presence of normal oxygen supply.

The high rate of glycolysis presumably provides several advantages for cell proliferation. First, increased glucose uptake allows cells to produce sufficient ATP. Because of the high rate of glycolytic activity, the percent of ATP produced from glycolysis could exceed that produced from oxidative phosphorylation [1]. Second and perhaps more importantly, glycolysis, as opposed to completely metabolism of glucose to water and CO_2 after TCA and oxidative phosphorylation, provides many intermediates for cell biosynthesis, including glycerol for lipids, ribose-5-phosphate for nucleotides, nonessential amino acids, and so on. Thus, high glucose uptake benefits the cancer cells both in bioenergetics and biosynthesis.

In addition to glucose, increased glutamine uptake is another tumor-specific metabolic alteration [8,9]. Glutamine is first converted to glutamate by glutaminase and then deaminated to produce α-ketoglutarate (α-KG) to enter the TCA cycle by either a transaminase or glutamate dehydrogenase. Glutamine plays several important roles in supporting cell growth and proliferation. First, glutamine serves as a carbon source and a nitrogen donor for energy production and biosynthesis. It was reported that glutamine could fully sustain the oxidative TCA cycle for energy production, even in the absence of glucose [10]. In proliferating cells, glutamine could contribute to citrate production and *de novo* lipogenesis through the reductive carboxylation of α-KG by isocitrate dehydrogenase under hypoxia and glucose starvation conditions [10–13]. Second, glutamine serves as an activator of mTOR1, which is a central cell growth controller, to promote cell growth. Increased intracellular glutamine could be used to facilitate the import of essential amino acids [14,15]. Third, glutamine takes part in the synthesis of glutathione, an endogenous antioxidant, to maintain the cellular redox homeostasis. Stimulation of glutamine uptake by p53 was shown to result in an increased ratio of reduced to oxidized glutathione (GSH/GSSG) and protect the cells against oxidative stress [16,17].

2 Mutations of metabolic genes in cancer

2.1 Mutations of *SDH* genes cause the accumulation of succinate

Succinate dehydrogenase (SDH) is involved in the TCA cycle and also participates in the electron transport chain as the respiratory complex II. SDH is composed of four subunits, which are named SDHA, SDHB, SDHC and SDHD, and is activated by SDH5 (also named SDHAF2) [18]. SDH catalyzes the conversion of succinate to fumarate with the concomitant reduction of ubiquinone to ubiquinol. The *SDH* gene was the first metabolic gene discovered to be mutated in human cancer, specifically in paragangliomas [19,20]. *SDH* mutations were also found in a number of other tumors, including gastrointestinal stromal tumors [21], renal cell tumors [22], childhood T-cell acute leukemia, pheochromocytoma and neuroblastomas [23]. Among all the *SDH* mutations, a high frequency of *SDH* germline mutations was identified in malignant extra-adrenal paragangliomas, and these mutations were associated with poor prognosis [24–26]. These studies suggest that *SDH* mutations offer a strong advantage for cancer cell proliferation. So far, there have been more than 650 reported cases of *SDH* mutations. In three cases of paragangliomatosis (HPGL) with *SDH* mutation, succinate accumulated to a high level of 364–517 μmol g^{-1} protein [27]. More recently, it was reported that either depleting *SDH* in mice or ectopic expression of tumor-derived SDH mutants resulted in the accumulation of succinate [28].

2.2 Mutations of *FH* genes in cancer cause the accumulation of fumarate

Fumarate hydratase (FH) is the enzyme next to SDH in the TCA cycle, and catalyzes the reversible hydration/dehydration of fumarate to malate. The human genome contains only one FH gene, which encodes two different forms of fumarate hydratase. The longer form localized in mitochondrion participates in the TCA cycle, and the short form localized in the cytoplasm is involved in the urea cycle and purine synthesis. Mutations in *FH* gene were first identified in inherited uterine fibroids, skin leiomyoma and papillary renal cell cancer by a combination of mapping methods [29]. *FH* mutations were also found in cerebral cavernomas [30], Leydig cell tumors [31] and ovarian mucinous cystadenoma with low frequency [32]. So far, *FH* mutations have been found in more than 300 cases. As with *SDH* mutations, *FH* mutations resulted in the accumulation of fumarate to a level as high as 417–688 μmol/g protein in hereditary leiomyomatosis and renal cell cancer [27]. The accumulation of fumarate was also observed in cells depleted for *FH* or expressing a tumor-derived FH mutant [28].

2.3 *IDH1* and *IDH2* are most frequently mutated metabolic genes in human cancers

IDH catalyzes the oxidative decarboxylation of isocitrate to produce α-KG. The IDH family includes three different isoforms: IDH1, IDH2 and IDH3. IDH1 is located in the cytosol and peroxisomes, while IDH2 and IDH3 are located in mitochondria. IDH1 and IDH2 use $NADP^+$ as a cofactor, while IDH3 uses NAD^+ as a cofactor in the TCA cycle for energy metabolism [33].

The *IDH1* mutation was first found in human glioblastoma multiforme by a cancer genome project in 2008 [34]. A short time later, another cancer genome project identified a mutation in the *IDH1* gene in leukemic cells from an acute

myeloid leukemia (AML) patient compared with his normal skin cells [35]. These two findings were quickly confirmed by multiple groups through direct sequencing of *IDH1* and its homologue *IDH2*. Mutations in *IDH1* and *IDH2* were found in 75% of grade 2 to 3 gliomas and secondary glioblastoma, and in about 20% of AML [36–48]. Following the discovery in glioma and AML, *IDH1* and *IDH2* mutations were also found in several other human tumors, including cartilaginous tumors (75%) [49–51], intrahepatic cholangiocarcinoma (10%) [52,53], thyroid carcinomas (16%) [54,55], and less frequently in prostate cancer, acute B-lymphoblastic leukemia, paragangliomas, colorectal carcinoma, and melanoma [52,56,57]. Thus, *IDH1* and *IDH2* represent the most frequently mutated metabolic genes in human cancer (Table 1).

The mutations of *IDH1* and *IDH2* in different types of human tumors share four unique biochemical features. First, *IDH1* and *IDH2* mutations are all heterozygous and there is no loss of heterozygosis of the wild-type copy. This is consistent with both a gain of function and dominant effect over the remaining wild-type allele. Second, nearly all *IDH1* and *IDH2* mutations predominantly target a single amino acid residue, Arg132 in IDH1 (to one of six amino acid residues: His, Cys, Leu, Ile, Ser, Gly, or Val), or the corresponding Arg172 in IDH2 (to one of four different residues: Lys, Met, Gly, and Trp), and Arg140 in IDH2 (to either Gln or Trp). Moreover, these three residues are located in the active sites of the enzymes, suggesting a direct impact of the mutations on the enzyme catalytic activity. Two additional mutations targeting IDH1, R100A and G98D, was also found to occur less frequently in adult glioma and in a colon cancer cell line [58]. Third, all *IDH1* and *IDH2* mutations in human tumors are found to be somatic mutations, with germline mutations rarely observed. Finally, *IDH1* and *IDH2* mutations occur in a mutually exclusive manner in most cases, and few tumors have been found to harbor mutations in both *IDH1* and *IDH2* genes [38]. This fact suggests that mutations targeting *IDH1* and *IDH2* share a common underlying biochemical mechanism.

2.4 Mutant IDH1 and IDH2 lose their normal activity to produce α-KG and gain a new activity of producing D-2-HG

Shortly after the first IDH1 mutation was reported, it was found that tumor-derived IDH1 mutants lost their normal activity to produce α-KG. This functional inactivation led to a decrease of α-KG and increase of HIF1a protein levels, possibly because of the inhibition of prolyl hydroxylase (PHD) [59]. Thus, loss of the normal function of producing α-KG is the first biochemical alteration of tumor-derived IDH1 and IDH2 mutants.

A subsequent study using metabolite profiling surprisingly found that the IDH1 mutant not only loses its normal activity in the production of α-KG, but also gains a new function: catalyzing the NADPH-dependent reduction of α-KG to D-2-hydroxyglutarate (D-2-HG, also known as *R*-2-HG) [60]. Further studies showed that all the tumor-derived mutants targeting three hot spots, Arg132 in IDH1 and Arg140 and Arg172 in IDH2, also gain this new activity [61–63]. In human glioma with IDH1/2 mutation, the level of D-2-HG accumulates as high as 5–35 μmol g^{-1} (or 5–35 mmol L^{-1}) [42]. In addition to glioma, D-2-HG accumulation has also been found in AML and enchondroma [50]. Taking advantage of such high D-2-HG levels, efforts

Table 1 Mutation of eight metabolic genes

Enzyme	Gene	Catalytic reaction	Tumor types	Mutation frequency
IDH1 (isocitrate dehydrogenase1)	*IDH1*	Isocitrate+$NADP^+$→ α-KG+NADPH+CO_2	Glioma	~75%
			AML	20%
			Enchondroma	84%
			Chondrosarcoma	56%
			Thyroid carcinomas	17%
			Cholangiocarcinoma	10%
IDH2 (isocitrate dehydrogenase2)	*IDH2*		Prostate cancers	2/79
			B-cell ALL	1/60
			Paragangliomas	1/131
			Colorectal carcinoma	2/180
SDH (succinate dehydrogenase)	*SDHA*	Succinate+ubiquinone→ fumarate+ubiquinol	Parganglioma	6/36
	SDHB		Merkel cell carcinoma	2/18
	SDHC		Phaeochromocytoma	5/22
	SDHD		GIST	6/38
	SDHAF2		Midgut carcinoid	2/18
FH (fumarate hydatase)	*FH*	Fumarate+H_2O→malate	Rebal cell carcinoma	1/3
			Lung adenocaicinoma	1.1%
			Melanoma	1/14
			Leiomyoma	1.3%

have been made to noninvasively detect the accumulation of D-2-HG in glioma patients by magnetic resonance spectroscopy techniques for tumor diagnosis and imaging [64, 65].

3 The mechanism of tumorigenesis linked to *SDH*, *FH* and *IDH* mutations

3.1 α-KG dependent dioxygenases are a large family of enzymes involved in diverse cellular processes

One emerging mechanism common to the tumorigenesis linked to mutations in all four metabolic enzymes is that they all lead to impaired activity of a family of enzymes collectively known as α-KG-dependent dioxygenases. In 1967, Hutton et al. [66] identified the first α-KG-dependent dioxygenase, which was named collagen prolyl hydroxylase (CPH). After this pioneering work, the α-KG-dependent dioxygenases have been established as a widely distributed family. Sequence homology analysis suggests that there are more than 60 members in humans [67,68]. The α-KG dependent dioxygenases catalyze the hydroxylation reactions with diverse substrates, including collagen, histones, transcription factors, alkylated DNA and RNA, lipids, antibiotics, and the recently discovered 5-methylcytosine of genomic DNA and 6-methyladenine of RNA [69,70]. In recent years, the biological roles of α-KG-dependent dioxygenases have been expanded to multiple pathways, such as CPHs in collagen stabilization and endostatin production, trimethyllysine hydroxylase in fatty acid metabolism, prolyl hydroxylases (PHDs) and factor inhibiting-HIF (FIH) in hypoxic signaling, AlkB homologs in DNA and RNA repair, histone lysine demethylases (KDMs) and DNA hydroxylases of ten-eleven translocases (TETs) for epigenetic regulation on histones and DNA (Table 2).

α-KG plays critical roles in multiple cellular metabolic pathways, such as TCA cycle, anaplerosis, amino acid and fatty acid synthesis, and hydroxylation of proteins and nucleic acids. In the hydroxylation reaction, α-KG serves as a co-substrate for all α-KG-dependent dioxygenases [70], which use both atoms of molecular oxygen for substrate hydroxylation. Both α-KG and O_2 are considered to be cosubstrates, with one oxygen atom attached to a hydroxyl group in the substrate (hydroxylation) and the other one taken up by α-KG, leading to the decarboxylation of α-KG and formation of carbon dioxide and succinate. All α-KG-dependent dioxygenases require Fe(II) as a cofactor. The last role of α-KG in supporting the α-KG-dependent dioxygenases appear to be impaired in cells with mutation targeting any of four metabolic enzymes.

3.2 SDH and FH mutants inhibit multiple α-KG dependent dioxygenases

In 1977, Raili et al. [71] found that succinate was a competitive inhibitor of α-KG and could suppress CPH, an α-KG dependent dioxygenase involved in collagen biosynthesis. Several years after the *SDH* mutation was reported, it was shown that the elevated level of succinate in cells caused by the inhibition of *SDH* could lead to the inhibition of PHDs, resulting in accumulation of both HIF-1α and HIF-2α [27]. This unveiled the first biochemical mechanism linking succinate to tumorigenesis. Another study further provided *in vivo* evidence for this model. Elevated levels of succinate and fumarate caused by *SDH* mutations in paragangliomas and *FH* mutations in renal cell cancer, respectively, lead to the accumulation of HIF-1α through the inhibition of PHD [27]. As expected, elevated HIF-1α is also observed in cells

Table 2 α-KG dependent dioxygenases and their substrates

Dioxygenase	Km for αKG		Substrate	Effect on substrate	Associated disease
CPH-I (collagen prolyl hydroxylase, type I)	22 μmol L^{-1}		Collagen	Stabilization	Scurvy
CPH-II (collagen prolyl hydroxylase, tyep II)	22 μmol L^{-1}		Collagen	Stabilization	Scurvy
PHD1 (proline hydroxylase domain protein 1)	60 μmol L^{-1}		HIF-2α	Degradation	?
PHD2 (proline hydroxylase domain protein 2)	60 μmol L^{-1}		HIF-1α	Degradation	Cancer
PHD3 (proline hydroxylase domain protein 3)	60 μmol L^{-1}		HIF-2α	Degradation	?
FIH (factor inhibiting HIF)	25 μmol L^{-1}		HIF-1α	Reducing p300 binding	?
		Ankyrin repeat domain (ARD)	NFκB	?	?
			Notch receptor	?	
			ASB4	?	
PhyH (phytanoyl coenzyme A hydroxylase)	?		phytanoyl coenzyme A		Refsum disease
FTO (fat mass and obesity associated)	?		DNA demethylation		Obesity
ABH1-8 (AlkB homologs)	?		DNA (and RNA?) demethylation		DNA repair
AlkB (*E. coli*)	1 μmol L^{-1}		DNA demethylation		
KDM/JMJD (JmiC domain proteins)	?		Histone demethylation		Gene regulation epigenetics
TET (Ten Eleven-translocation)	?		Hydroxylation of 5-mC		Gene regulation epigenetics

with mutations of SDH or FH.

The KDMs and TET DNA hydroxylases are two sub-families of α-KG-dependent dioxygenases that control the epigenetic modifications in the cells. A recent study showed that both succinate and fumarate inhibit KDM from human and *C. elegans*. The IC_{50} of succinate and fumarate of human KDM4A are 0.8 and 1.5 mmol L^{-1}, respectively [28]. Knocking down *SDH* or *FH* or overexpression of the tumor-derived SDH or FH mutant significantly increased genome-wide histone and DNA methylation [28]. Furthermore, two DNA methylation profiling studies found that *SDH* mutations in gastrointestinal stromal tumors, paragangliomas and pheochromocytomas were associated with genomic hypermethylation [72,73]. In addition, decreased levels of 5-hydroxymethylcytosine were associated with *SDH* mutations in gastrointestinal stromal tumors [74]. Taken together, the *in vivo* evidence strongly supports that mutation in *FH* or *SDH* results in global genome changes in DNA methylation, likely due to inhibition of TET proteins by fumarate or succinate, thus leading to epigenetic alterations in cancers. These studies raise an exciting possibility of treating cancers by interfering with epigenetic pathways.

3.3 IDH mutants inhibit α-KG-dependent histone and DNA demethylases

The oncometabolite of D-2-HG, produced by IDH mutants, acts as an antagonist of α-KG to inhibit multiple α-KG dependent dioxygenases. However, not all α-KG dependent dioxygenases are equally inhibited by D-2-HG. A systematic *in vitro* enzymatic study showed that the histone H3K9/H3K36 demethylases KDM4A/JMJD2A and KDM4C/JMJD4C are most sensitive to a low IC_{50} of 24 and 79 μmol L^{-1}, respectively [75]. Three *in vivo* studies confirmed this finding in cultured cells, human tumor samples, and mouse models. First, elevated levels of multiple histone methylation markers were observed in cells expressing tumor-derived IDH1/2 mutants and gliomas with an $IDH1^{R132H}$ mutation [63]. Second, IDH mutation is associated with a block to differentiation and repression of lineage-specific differentiation genes through the inhibition of KDM4C [76]. Third, increased levels of histone methylation markers, including trimethyl H3K4, H3K9, H3K27, and H3K36 and dimethyl H3K79, are observed in $IDH1^{R132H}$ knock-in cells [77] and an $IDH1^{R132H}$ conditional knock-in mouse model [78].

The TET family of DNA hydroxylases catalyzes three sequential oxidative reactions, first converting 5-methylcytosine (5mC) to 5-hydroxylcytosine (5hmC), then to 5-formaylcytosine (5fC), and finally to 5-carboxylcytosine (5caC), leading to DNA demethylation [79–83]. D-2-HG directly inhibits TET activity *in vitro*, and the expression of tumor-derived IDH1 or IDH2 mutants also inhibits TET-induced 5mC hydroxylation in transfected cells [61,63]. Importantly, genetic evidence supports TET DNA hydroxylase as the major target of IDH1/2 mutations. First, a promoter methylation analysis study demonstrated that a subset of glioblastoma, a proneural subgroup that is tightly associated with IDH1 mutations, shows a distinct CpG island methylation phenotype (G-CIMP) [84]. A similar DNA hypermethylation phenotype is observed in AML with *IDH1/2* mutation [61]. Recently, two genetic models, IDH1(R132H/WT) knock-in cells and mice, also showed global DNA hypermethylation [77,78]. These findings suggest a causal link between *IDH1/2* mutation and elevated DNA methylation. Second, mutations of *TET2* occur in a mutually exclusive manner with *IDH1/2* mutations, indicating a common pathogenesis caused by mutation in *TET2* or *IDH1/2* in AML [61]. Third, stable expression of an IDH1 mutant in primary human astrocytes induces extensive DNA hypermethylation and reshapes the methylome in a fashion that mirrors the changes observed in G-CIMP-positive lower-grade gliomas that have frequent IDH mutations [85]. Collectively, these studies establish a model in which TET hydroxylases are the major targets of *IDH1/2* mutations to promote tumorigenesis.

3.4 Succinate, fumarate and D-2-HG are structurally similar to and act as antagonists of α-KG

Structural studies have revealed a conserved coordination between α-KG-dependent dioxygenases and their cofactor and cosubstrate. In the catalytic core of α-KG-dependent dioxygenases, α-KG uses two oxygen atoms from the α-keto carboxyl end, one from its C-1 hydroxyl group and the other from its C-2 ketone group, to coordinate with Fe(II), and two other oxygen atoms from the acetate end to interact with conserved amino residues in the dioxygenases. Structural analysis shows that D-2-HG is similar to α-KG except for the oxidative state at the C-2, whereby the ketone group is replaced by a hydroxyl group in D-2-HG (Figure 1). Likewise, both succinate and fumarate are structurally similar to α-KG, with the exception that the ketone group at the C-2 position in α-KG is missing in succinate and fumarate (Figure 1). Thus, these structural similarities suggest that succinate, fumarate and D-2-HG may act as competitive inhibitors of α-KG to interfere with the function of α-KG-dependent dioxygenases. This hypothesis was supported by a series of experiments both *in vitro* and *in vivo* [27,63,71–74,84]. Moreover, structural analyses have shown that succinate and fumarate could bind the α-KG-dependent dioxygenases in their catalytic core just like α-KG, such as FIH and AlkB [70,86]. Additionally, D-2-HG binds KDM7A in a configuration similar to α-KG [63]. Thus, these results provide direct evidence supporting succinate, fumarate, and D-2-HG as antagonists of α-KG in the cell to inhibit α-KG-dependent dioxygenases.

4 Conclusion and perspectives

During the past ten years, researchers have shown increasing interest in metabolism alterations in tumorigenesis, fueled in part by the discovery of metabolic gene mutations in cancer. Common metabolites not only can regulate enzyme activity through classical allosteric effects, but also influence multiple cellular processes through global effects, such as inhibition of the dioxygenase family. As discussed above, three oncometabolites, D-2-HG, succinate and fumarate, act as antagonists of α-KG and inhibit a large number of α-KG-dependent dioxygenases to control a diversity of cellular activities, including hypoxia response and epigenetics regulation (Figure 2). One may also speculate that many other metabolites serve as signaling molecules to influence whole cell and body physiology. Therefore, proteins/enzymes that are regulated by metabolites can be considered as cellular sensors for metabolic status, thereby functioning to coordinate global cellular activity/function with metabolic status.

The realization that metabolites can have global regulatory effects on cells not only significantly advances our understanding of the intricate regulatory network between small molecules (metabolites) and macromolecules (protein, RNA, and DNA), but also provides exciting leads for future therapeutic intervention for disease treatments. By nature, small molecules are much easier to be produced, optimized

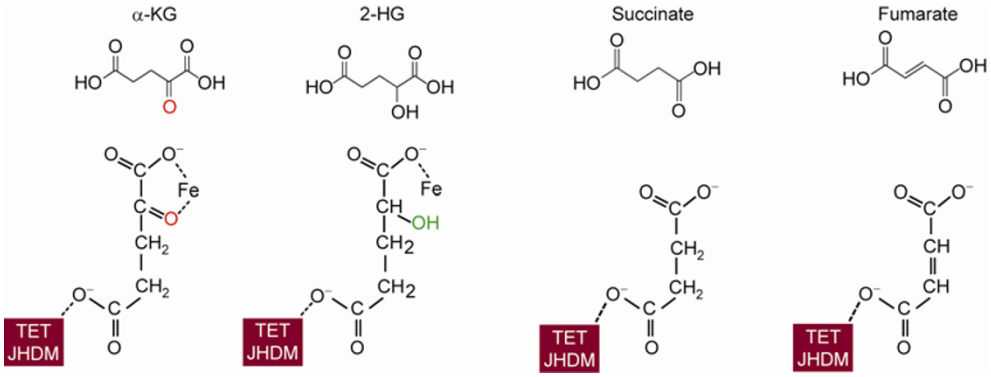

Figure 1 Structural comparison of fumarate, succinate, α-KG, and D-2-HG. The oxygen in the ketone group at the C-2 position of α-KG is responsible for the coordination of Fe(II) in α-KG-dependent dioxygenase. All three oncometabolites, D-2-HG, fumarate and succinate, share structural similarity with α-KG, except for the oxidative state at the C-2 position. The 2-ketone group at the C-2 position in α-KG is replaced by a hydroxyl group in D-2-HG and is absent in fumarate and succinate.

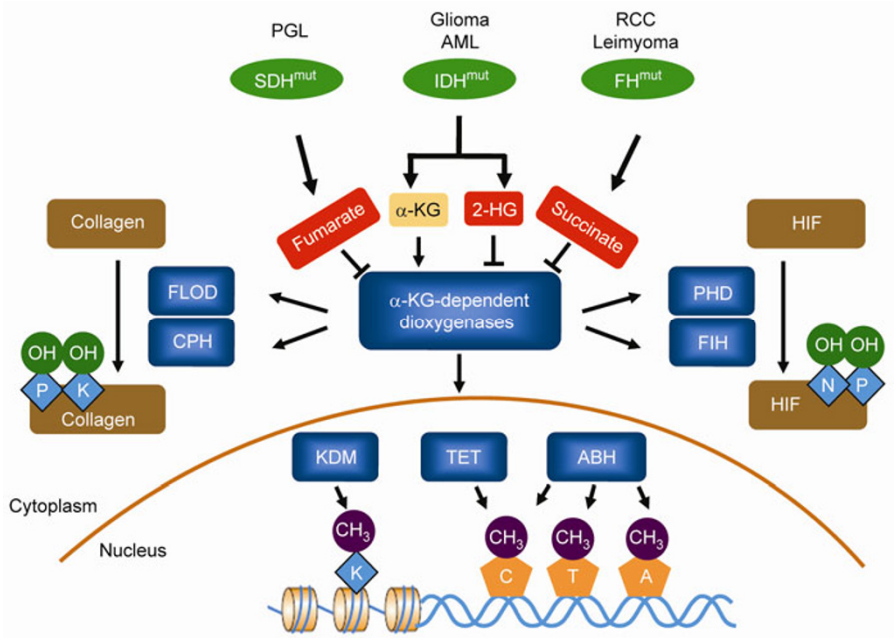

Figure 2 *IDH1/2* mutations alter epigenetic regulation by inhibiting both histone and DNA demethylation. Mutations of *IDH1/2*, *SDH* and *FH* lead to the accumulation of D-2-HG, fumarate and succinate, respectively, which can function as α-KG antagonists to broadly inhibit α-KG-dependent dioxygenases, including the JMJD family KDMs and the TET family of 5mC hydroxylases. PGL, paragangliomatosis; AML, acute myeloid leukemia; RCC, renal cell carcinoma.

by modification, and delivered into the human body. The mechanistic understanding of metabolites in cellular regulation will facilitate the use of metabolites or their analogs for treatment of human disease.

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Biographical Sketch

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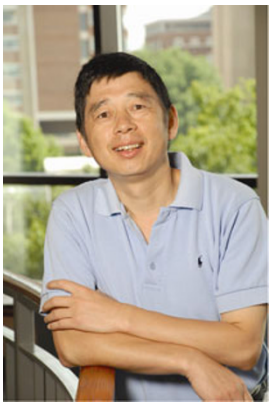

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