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Review Article

Targeting 2-oxoglutarate dehydrogenase for cancer treatment

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Abstract: Tricarboxylic acid (TCA) cycle, also called Krebs cycle or citric acid cycle, is an amphoteric pathway, contributing to catabolic degradation and anaplerotic reactions to supply precursors for macromolecule biosynthesis. Oxoglutarate dehydrogenase complex (OGDHc, also called α-ketoglutarate dehydrogenase) a highly regulated enzyme in TCA cycle, converts α-ketoglutarate (αKG) to succinyl-Coenzyme A in accompany with NADH generation for ATP generation through oxidative phosphorylation. The step collaborates with glutaminolysis at an intersectional point to govern αKG levels for energy production, nucleotide and amino acid syntheses, and the resources for macromolecule synthesis in cancer cells with rapid proliferation. Despite being a flavoenzyme susceptible to electron leakage contributing to mitochondrial reactive oxygen species (ROS) production, OGDHc is highly sensitive to peroxides such as HNE (4-hydroxy-2-nonenal) and moreover, its activity mediates the activation of several antioxidant pathways. The characteristics endow OGDHc as a critical redox sensor in mitochondria. Accumulating evidences suggest that dysregulation of OGDHc impairs cellular redox homeostasis and disturbs substrate fluxes, leading to a buildup of oncometabolites along the pathogenesis and development of cancers. In this review, we describe molecular interactions, regulation of OGDHc expression and activity and its relationships with diseases, specifically focusing on cancers. In the end, we discuss the potential of OGDHs as a therapeutic target for cancer treatment.

Keywords: α-ketoglutarate dehydrogenase complex, 2-oxoglutarate dehydrogenase, tricarboxylic acid cycle, cancer metabolism, reactive oxygen species

Introduction

OGDH complex (OGDHc) is a rate-limiting enzyme in the tricarboxylic acid (TCA) cycle, catalyzing α-ketoglutarate (αKG) to yield succinyl-Coenzyme A (succinyl-CoA) and NADH. OGDHc is located in the matrix of mitochondria where it is binds to Complex I of electron transport chain (ETC) at the matrix side of the inner membrane [1, 2]. The product, succinyl-CoA, can be used as the substrate for succinyl-CoA synthetase (SCS) within TCA cycle, but it also serves as the major precursor for heme biosynthesis [3]. Interestingly, the downstream enzyme succinate dehydrogenase (SDH, also called succinate-coenzyme Q reductase) in TCA cycle, is also an integral component of Complex II in ETC (**Figure 1**). These characteristics manifest the critical role of OGDHc in regulating mitochondrial reactive oxygen species (ROS) production and cellular redox homeostasis [4].

In TCA cycle, αKG, also referred to 2-oxoglutaric acid, is generated from isocitrate by isocitrate dehydrogenases (IDHs) via oxidative decarboxylation, while αKG also can be produced anaplerotically from glutamate through the glutaminolysis pathway to provide substrates for energy production and macromolecule syntheses [5, 6]. Normally, αKG is oxidatively decarboxylated to succinyl-CoA and CO_2 via OGDHc. Due to the need for fast growth and proliferation, how-

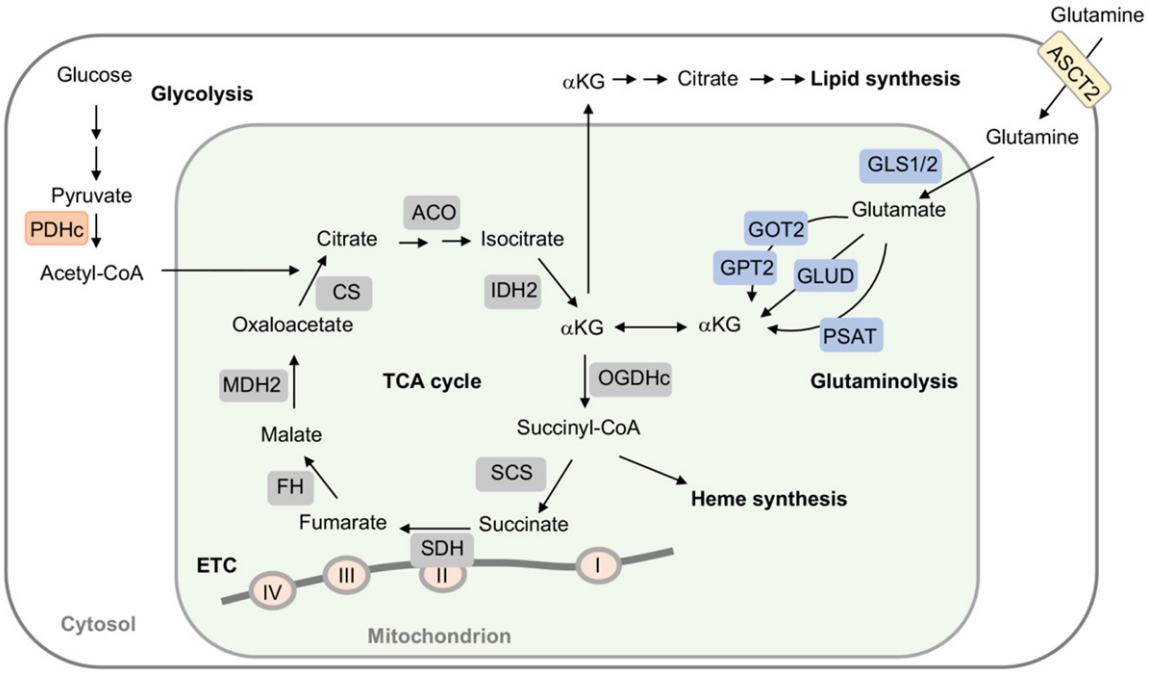

Figure 1. The role of OGDH and αKG in bioenergy synthesis. αKG is generated from glucose-derived citrate, namely glycolysis and glutaminolysis. Glutaminolysis initiates from deamination of glutamine via glutaminases (GLS1/GLS2) to produce glutamate and ammonia. Glutamate is then converted to αKG by glutamate dehydrogenase (GLUD) or through transamination by glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase 2 (GPT2), as well as, phosphoserine transaminase (PSAT). αKG is oxidatively decarboxylated by 2-oxoglutarate dehydrogenase complex (OGDHc) to succinyl-CoA and CO_2, followed by succinyl-CoA synthetase (SCS) converting to succinate. Succinyl-CoA is also forwarded to heme synthesis. Succinate is then oxidized to fumarate by succinate dehydrogenase (SDH). SDH is a part of both tricarboxylic acid (TCA) cycle and electron transport chain (ETC), where SDH is a component of Complex II. However, when pyruvate dehydrogenase complex (PDHc) activity is impeded, αKG is converted to citrate for lipid generation. ACO, aconitase; CS, citrate synthase; FH, fumarate hydratase; IDH2, isocitrate dehydrogenase 2; MDH2, malate dehydrogenase 2.

ever, glutamine is highly demanded and glutaminolysis process is greatly enhanced to support αKG supply for energy need and macromolecule biosynthesis in cancer cells [7]. Under hypoxia, a decrease of glucose-derived citrate and inhibition of OGDH activity cause αKG buildup, which in turn reverses IDH reaction and results in citrate accumulation and following lipid synthesis [8]. Thus, cancer cells greatly rely on reductive glutamine metabolism for lipid synthesis [9]. In addition, dysregulation of OGDH amplifies reductive carboxylation under hypoxia and defective IDH activity leading to accumulation of αKG and oncometabolites, such as 2-hydroxyglutarates (2-HG), fumarate, malate, enhanced hypoxia inducible factor-1α (HIF-1α) stabilization [10-12], and impaired DNA methylation to remodel intracellular environments optimal for cancer growth [11, 13-15]. These results signify hypoxia to drive reductive glutamine metabolism [8]. Accordingly, OGDHc operates at the intersection connecting bioenergy synthesis and anaplerotic pathways in metabolism. Several human diseases such as cancers and neurodegeneration have been linked to dysregulation of OGDHc activity [16, 17].

Although the precise mechanisms of OGDHc-regulated redox homeostasis and glutamine metabolism in cancer pathogenesis and progression remains fully understood, emerging data suggest a crucial role of OGDHc in cancer development. Herein, this review highlights the functional features of OGDHc in cancer development in respect to relevant metabolic pathways to delineate its potentials as a target in cancer therapy.

OGDH complex

The OGDH complex (OGDHc) is composed of OGDH (E1, also called α-ketoglutarate dehydro-

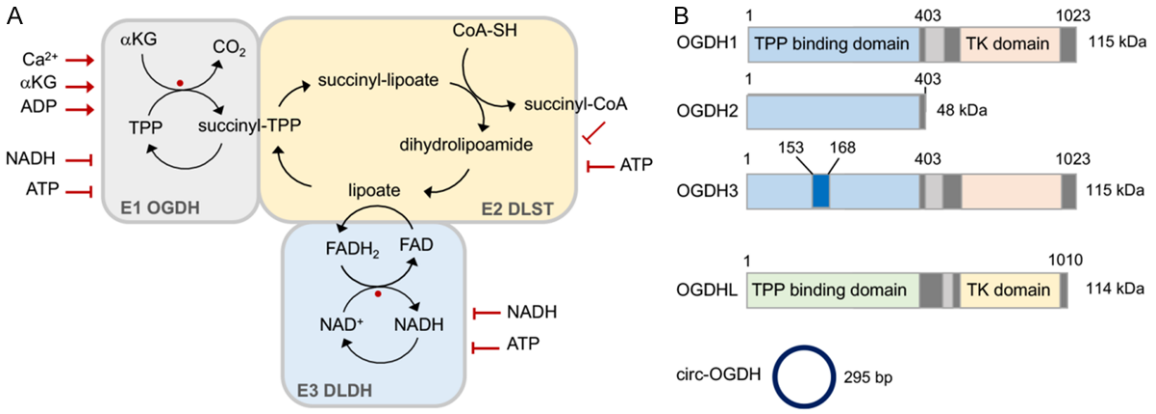

Figure 2. Regulation of OGDH complex. A. OGDH complex comprises three components, 2-oxoglutarate dehydrogenase (E1 OGDH), dihydrolipoamide succinyltransferase (E2 DLST), and dihydrolipoamide dehydrogenase (E3 DLDH), carrying out the coupled reaction to decarboxylate α-ketoglutarate to succinyl-CoA. E1 OGDH subunit is α-ketoglutarate decarboxylase that uses thiamine pyrophosphate (TPP) as a cofactor to decarboxylate α-ketoglutarate (αKG) for CO_2 production and succinylation of TPP. The succinyl-moiety is then transferred to lipoamide in the E2 DLST subunit where yields succinyl-CoA and dihydrolipoamide. The dihydrolipoamide is re-oxidized by E3 DLDH subunit. Here, free electrons are transferred from FAD to reduce NAD^+ to generate NADH. OGDH complex contains two susceptible sites for ROS generation (marked by red spots). All of the 3 subunits can be S-glutathionylation. Activity of OGDH complex is regulated by TPP, lipoic acid, CoA, FAD, NAD^+, succinyl-CoA, and ROS. The susceptible target by ROS is the prosthetic lipoic acid of the E2 DLST subunit. B. The isoforms of OGDH, OGDH-like (OGDHL), and circ-OGDH. There are three major variants of OGDH in human, OGDH1, OGDH2 and OGDH3. All three OGDH isoforms contain TPP binding domain. OGDH2 lacks of transketolase (TK) domain. Circ-OGDH is the product of reverse splice of *OGDH* gene, 295 bp.

genase; KGDH), dihydrolipoamide *S*-succinyltransferase (DLST, E2), and dihydrolipoamide dehydrogenase (DLDH, E3) (**Figure 2A**). E1 and E2 are unique for OGDHc, but E3 is also a part of pyruvate dehydrogenase complex (PDHc), branched-chain ketoacid dehydrogenase complex (BDKDHc), α-ketoadipate dehydrogenase (KADH), and glycine cleavage enzyme [18]. OGDH-derived succinyl-CoA is converted into succinate followed by oxidation to generate fumarate via SCS and SDH. SDH is also functions as a component of Complex II of ETC (**Figure 1**). OGDH is encoded by *OGDH* gene. In human, there are three major splice variants of OGDH gene, variant 1 (OGDH1, 115 kDa, here is referred to OGDH), variant 2 (OGDH2, 48 kDa) and variant 3 (OGDH3, 115 kDa) (**Figure 2B**). OGDH1 and OGDH3 share 99% identity except at amino acid fragment 153-169, while OGDH2 shares with OGDH1 identical amino acid 1-403 [8]. OGDH2 is also able to associate with E2 DLST and E3 DLDH and serves as an OGDH-similar function in TCA cycle [8]. The function of OGDH3 remains further investigations. Circular RNA circ-OGDH, a reverse splicing of *OGDH* gene, also has been identified [19, 20]. The amino acid residue 320 of *OGDH* gene is required for OGDH activity [21]. The c.959A>G variant in OGDH gene leads to an amino acid change (p.Asn320Ser), causing a severe loss of OGDH activity. Patients with the mutation exhibited systemic delay in development, elevated serum lactate levels, ataxia, and seizure [21]. The deficiency of E3 subunit DLDH is rare and has been reported as an autosomal recessive genetic disorder [22].

Within the OGDHc, thiamine pyrophosphate (TPP) serves as a cofactor to reduce NAD^+ to NADH and catalyzes the reaction between αKG and CoA-SH to generate succinyl-CoA and CO_2 along TCA cycle flux. Therefore, the complex activity is regulated by TPP, lipoic acid, CoA, FAD, NAD^+, succinyl-CoA, and ROS [17]. The reaction initiates from E1 subunit utilizing the cofactor TPP to decarboxylate αKG to generate a succinyl-TPP intermediate, which in turn proceeds with reductive succinylation on the lipoyl group of the E2 subunit DLST. Then, the E2 subunit serves as a dihydrolipoamide succinyltransferase to transfer the succinyl group to CoA to produce succinyl-CoA and dihydrolipoamide. E3 DLDH subunit thereafter uses FAD to oxidize dihydrolipoamide to produce a disulfide bond for further catalyzing events. During this process, NAD^+ is used to oxidize $FADH_2$

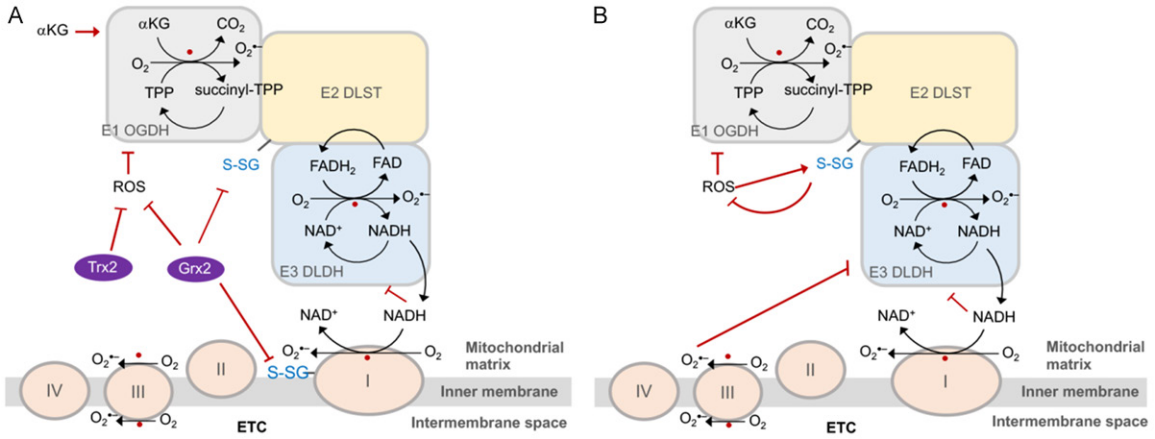

Figure 3. The role of OGDH complex in redox homeostasis. A. OGDH complex (OGDHc) is a potential ROS generator. E1 OGDH and E3 DLDH are two generation sites for ROS production (marked by red spots) within the OGDHc. E3 DLDH-derived NADH promotes ROS generation by Complex I located in electron transport chain (ETC). The glutathionylation (-S-SG) of OGDHc and Complex I enhances ROS generation, which can be suppressed by glutathione reductase 2 (Grx2) and thioredoxin 2 (Trx). OGDHc-generated ROS is also increased by α-ketoglutarate (αKG). B. OGDHc contributes to antioxidant defense. ROS generated from E3 DLDH can inhibit E1 OGDH and itself activities. ROS-enhanced glutathionylation of E2 DLSH protects peroxidative effects by ROS. The capability of ROS generation of E3 DLDH are regulated in response to cellular oxidative status, and thus, E3 DLDH-generated ROS is inhibited when a higher level of Complex III-derived ROS occurs.

back to FAD for NADH generation (**Figure 2A**). Similar to OGDHc, the catalytic mechanisms of PDHc and BDKDHc also utilize E1 and E2 subunits to specifically decarboxylate their respective substrates while the E3 subunit shares the same catalyzing reaction [18, 23]. The lipoylation of OGDH thus controls the partition of carbon flux into TCA cycle [24]. Besides, calcium also acts as a key regulator of OGDH activity, which lowers the apparent Km of OGDH enzyme [25, 26]. However, OGDH activity is inhibited under high calcium concentrations [27]. Similarly, the impact by high levels of mitochondrial calcium also occurs in PDH and IDH [25]. Several splice variants of OGDH with a Ca^{2+}-insensitive characteristic have been identified [28], but their functional alterations and following impacts remain further investigations. Moreover, the sensibility of OGDH to Ca^{2+} relies on the concentration of NADH and ATP/ADP ratio, in a dose dependent manner to attenuate the stimulatory effect by Ca^{2+} [29].

Interestingly, translocation of TCA intermediates and enzymes, and operation of a TCA cycle subnetwork have been observed within the nucleus [30-32], but the function of OGDH in the nucleus require more intensive studies. Current studies suggest that nuclear OGDH can bind to the promoter regions, where it facilitates succinyl-CoA binding to lysine acetyltransferase 2 (KAT2A) and meanwhile, KAT2A exerts a succinyltransferase activity to succinylate histone H3 leading to epigenetic regulation in tumor cell proliferation [32, 33]. In addition, OGDH also regulates the succinylation of histone H4 [34]. Whether DLST and/or DLDH coexist with OGDH and form a functional complex for histone succinylation and following epigenetic regulation remain further studies.

The role of OGDH in redox homeostasis

OGDHc as a ROS generator

For a long time, the complexes of the respiratory chain are regarded as the major site of ROS production in the mitochondria. However, recently studies have demonstrated that OGDHc is an important source of mitochondrial ROS generation [4, 35, 36]. E3 DLDH subunit is the main site to generate ROS, whereas E1 OGDH subunit generates fewer ROS [4, 37-39]. When NAD^+/NADH ratio is low, $FADH_2$ within the E3 DLDH subunit can be oxidized by O_2, generating a semiquinone ($FADH^•$) and superoxide ($O_2^{•-}$) [37-39]. Besides, OGDHc can generate superoxide and hydrogen peroxide (H_2O_2) in response to high levels of αKG and free CoA [36-38] (**Figure 3A**).

The lipoyl moiety of E2 DLST enzyme can be glutathionylated, which inhibits the enzyme activity and protects lipoic acid from modification by the electrophilic lipid peroxidation [4, 40, 41]. Earlier studies showed that the glutathionylation of OGDHc and Complex I were down-regulated by glutathione reductase 2 (Grx2) [4, 44, 45]. *In vivo* studies further showed that Grx2 deficiency increased the glutathionylation of OGDHc and PDHc in accompany with decreased ROS generation from the enzymes [42]. Although, the glutathionylation of E2 DLST subunit can protect E1 subunit from irreversible oxidation, glutathione administration was shown to amplify $O_2^{\bullet-}/H_2O_2$ formation from E3 DLDH enzyme and decrease NADH production [4]. In addition, thioredoxin 2 was shown to protect OGDHc from self-inactivation in a low NAD^+ status [43]. Moreover, early studies suggested that OGDHc interacts with Complex I of ETC, allowing a direct transfer of NADH to NADH-oxidation site of Complex I [2, 46, 47] (**Figure 3A**). Provocation of ROS due to oxidative metabolism of αKG accumulation was also confirmed in fumarate hydratase-deficient cancer cells [48]. The results suggest that OGDHc potentially contributes to mitochondrial electron leakage and following ROS production along its catalytic reactions and the potential greatly relies on the cellular redox status.

OGDHc contributes to antioxidant defense

As early in 1990s, a decline of OGDH activity has been identified in some neurodegenerative diseases such as Alzheimer's and Huntington diseases [16, 49, 50]. The deficiency of OGDH function in neurodegenerative diseases is highly associated with oxidative stress, indicating OGDH as a critical regulator in cellular redox homeostasis. In early studies, exposure of cardiac mitochondria to HNE (4-hydroxy-2-nonenal), a fragmentation product of lipid peroxidation, was shown to impair OGDH and PDH activity and suppress the NADH-dependent state III respiration, while other mitochondrial dehydrogenases and complexes of ETC were not affected [51]. The target by HNE attack was attributed to the prosthetic lipoic acid, which covalently binds to the E2 subunit within OGDHc and PDHc [52]. Apparently, OGDH is the most vulnerable target in response to oxidative stress within mitochondria [53]. Furthermore, H_2O_2 was shown to directly interact with the sulfhydryl groups of OGDH, modulating its glutathionylation status and enzymatic activity [40, 54]. During this modification process, lipoic acid requires cofactor covalently to attach to the enzyme and the glutathionylation of OGDH turn out to protect the prosthetic lipoic acid from peroxidation modification [4, 40]. The results manifest a regulatory loop of OGDHc in antioxidant defense involving the modification of the prosthetic lipoic acid in response to cellular redox status. In spite, the E3 DLDH subunit of OGDH is a flavoenzyme responsible for the transfer of reducing equivalents from lipoic acid to NAD^+ and contributes to most of ROS and H_2O_2 generation of OGDHc [36-38]. DLDH is vulnerable to peroxides; for example, inactivated by Complex III-derived H_2O_2 [55] (**Figure 3B**). Therefore, DLDH can induce or attenuate ROS production, depending on oxidative stress conditions. Aconitase is the most ROS-sensitive enzyme in TCA cycle [56], and its inhibition greatly interrupts the flux from pyruvate to αKG, thereby modulating OGDHc activity that limits NADH production and consequently ROS generation.

Several signaling pathways have been reported to participate in OGDH mediated redox homeostasis including mammalian target of rapamycin complex 1 (mTOR) and sirtuins (Sirt) [57]. In the crosstalk between mTOR and OGDH, a low dose of ROS exposure activates mTOR signaling, whereas high concentrations or long-term ROS treatment decreases mTOR activity [58]. The depletion of thiamine, a cofactor of OGDHc and PDHc, caused metabolic dysregulation and growth arrest in breast cancer and leukemia cells, which could be completely reversed by rapamycin treatment [59]. The results suggest that mTOR is involved in OGDH-related metabolic networks. A decrease of cellular αKG levels was shown to enhance the sensitivity of cancer cells to p53 inhibitor nutlin-3a-induced apoptosis, while the add-back of αKG analog dimethyl-αKG and genetic knockdown of OGDH rescued the cell viability [60]. Nardilysin, a metalloendopeptidase, is required for OGDH activity due to its facilitation on the folding of OGDH protein. A loss of nardilysin or OGDH resulted in αKG accumulation and consequently activated mTOR signaling and suppressed the autophagic process, while rapamycin treatment and a blockade of autophagy significantly ameliorated the neurodegeneration by nardily-

sin mutation [61]. The results highlight that mTOR serves as a downstream molecule of OGDH and its activity contributes to autophagic pathway. Genetic knockdown of OGDH-like (OGDHL), a variant of OGDH, has been shown to activate mTOR signaling pathway and consequently induce lipogenesis [62]. Sirt-5, was shown to repress cell growth and migration in gastric cancer by inhibiting OGDH activity due to its physical interaction with OGDH to impede the succinylation of OGDH [57].

The role of OGDHc and αKG in glutamine metabolism

Glucose and glutamine are the major nutrients for proliferation of mammalian cells, providing carbon and nitrogen for anabolism and macromolecule biosynthesis [64]. Cancer cells have a high demand of glutamine to meet the need for rapid growth and proliferation. Some cancers cells even require oncogene-dependent supplementations of glutamine in the culture systems [64]. In addition to glucose, glutamine also substantially contributes to citrate supply for fatty acid and glycerolipid synthesis in cancer cells [7, 65]. αKG from glutamine metabolism can be integrated into TCA cycle flux either for oxidative decarboxylation forward to ATP generation or through reductive TCA cycle for *de novo* lipogenesis or metabolite production [5, 66]. Accumulation of cellular αKG generally reflects an enhanced OGDHc activity and/or glutamine metabolism, namely glutaminolysis [5, 6].

Glutaminolysis is a catabolic process of glutamine to generate TCA cycle metabolites, initiating from deamination of glutamine via glutaminases (GLS1/GLS2), which produces glutamate and ammonia. Glutamate is then converted to αKG via two different pathways. The first pathway is accomplished by glutamate dehydrogenase (GLUD) and the second pathway is mediated through several transaminases, including glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), and phosphoserine transaminase (PSAT). The reaction by GLUD generates ammonium and NADH or NADPH, whereas the pathway by transaminases promotes the yield of some nonessential amino acids including asparate, alanine, and phosphoserine. Among these reactions, αKG serves as a resource for the anaplerotic reactions in TCA cycle [5, 6] (**Figure 1**). Thus glutamine metabolism provides the substrates for ATP generation through the respiration chain and for the synthesis of nucleotides, amino acids, lipids, macromolecules. In contrast to normal cells, cancer cells display elevated ROS production and moderate ROS levels tend to promote cancer cell proliferation, while excessive ROS might cause oxidative damages [67]. Glutaminolysis also functions in regulating redox homeostasis via the production of glutathione and NADPH to scavenge ROS. Along the TCA flux, αKG can be metabolized to fumarate, which directly interacts with glutathione peroxidase 1 (GPx1) to activate its activity, but also can trigger Nrf2 antioxidant signaling for ROS scavenging [68, 69].

The critical role of OGDHc in cancer growth

OGDH in cancer growth

Due to the specific metabolic plasticity, cancer cells exhibit a great reliance on OGDH activity [70]. In gastric cancer patients, positive correlations between OGDH activity and several clinicopathological parameters were observed and the underlying mechanisms were attributed to enhanced mitochondrial function and Wnt/β-catenin signaling [63]. Succinyl phosphonate, an OGDH inhibitor, was shown to significantly affect cell cycle and suppress cell migration and cell viability in AGS and BGC823 gastric cancer cells [57]. Moreover, some cancer cells even displayed a high sensitivity to OGDH depletion [71]. Dysregulation of OGDH activity has been reported to contribute to fumarate accumulation in fumarate dehydratase-deficient cancer cells, which turns out to stabilize HIF-1α from degradation and enhance ROS generation [48]. Accordingly, OGDH-dependent cancer cells tend to exhibit lower asparate and malate-asparate shuttle activity [72]. Mutations in the catalytic subunit of phosphatidylinositol 3-kinase (PI3KCA) have been reported in a variety of cancer types including breast, colorectal endometrial, and lung cancer and the PI3KCA mutant cancer cells showed a great reliance on OGDH activity for their proliferation and growth [71, 73]. Downregulation of OGDH by siRNA and αKG supplementation depleted cellular asparate levels, and desensitized malate-asparate shuttle, which in turn impeded cytoplasmic NAD^+ generation leading to disrupted NAD^+/NADH homeostasis [71] (**Figure 4**).

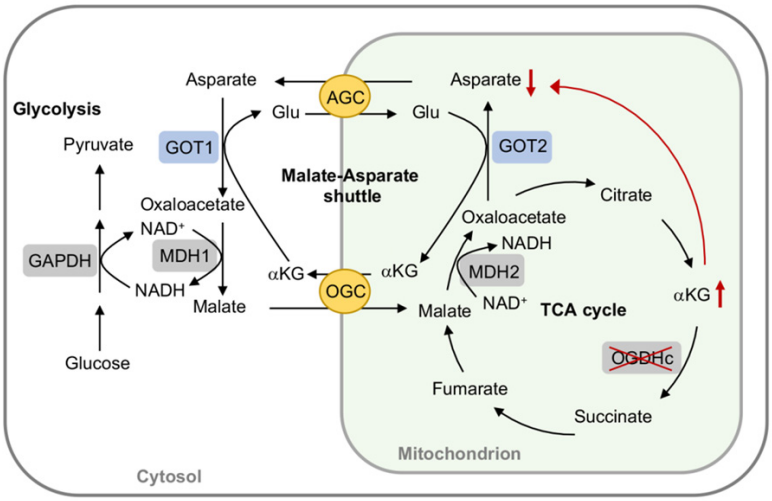

Figure 4. OGDH regulates malate-asparate shuttle activity. In OGDH-dependent or mutated catalytic subunit of phosphatidylinositol 3-kinase cancer cells, a lower OGDH activity leads to α-ketoglutarate (αKG) accumulation, which in turns to ameliorate the asparate and malate-asparate shuttle activity due to the depletion of asparate levels and disruption of NAD^+/NADH homeostasis. AGC, asparate/glutamate carrier; GAPDH, glyceraldehyde-3-P-dehydrogenase; Glu, glutamate; GOT1/2, glutamate oxaloacetate transaminase 1/2 (asparate transaminase); MDH1/2, malate dehydrogenase 1/2; OGC, oxoglutarate/malate carrier.

CircRNA circ-OGDH (hsa_circ_0003340), located at chr7: 44684925-44687358, encoding a length of 295 bp, is produced by reverse splicing of *OGDH* gene. Circ-OGDH has been reported to act as an oncogene in esophageal squamous cell carcinoma [19]. Circ-OGDH affects cancer cell proliferation, migration, and invasion, by operating at glutamine metabolism for αKG and ATP production and by downregulating miR-615-5p to increase PDX1 (pancreatic and duodenal homeobox) expression [20]. In addition, circ-OGDH was shown to decoy miR-564 to promote cell invasion and proliferation by upregulating TPX2 (targeting protein for Xenopus kinesin like protein 2) expression [19]. Circ-OGDH displays a similar function to OGDH by operating glutamine metabolism and fuel utilization to promote cancer cell growth. In addition, circ-OGDH serves an up-regulator of oncogenes via modulating of specific miRNA levels.

OGDHL in cancer growth

OGDHL encoded by *OGDHL* gene is a variant of OGDH, sharing the similar nucleotide sequences with OGDH. Expression of OGDHL is tissue-specific mainly in the brain and liver [74, 75]. Emerging data indicated that OGDHL is a tumor suppressor. Downregulation of OGDHL has been shown to contribute to the onset and progression of several cancers including colorectal, liver, pancreatic cancer, hepatocellular carcinoma, and cervical cancer cells [62, 76-80]. The lower OGDHL expression in the cancer cells was attributed to aberrant promoter hypermethylation and DNA copy deletion of OGDHL [62, 81], as well as, due to alternative splicing and downregulation by certain microRNA such as upregulated expression of miR-193a-59 in pancreatic cancer [79, 80]. miR-193a-5p functions to promote pancreatic cancer cell migration and invasion by targeting serine/arginine-rich splicing factor 6 (SRSF6) for OGDHL alternative splicing and downregulation [80]. Furthermore, OGDHL is also a target gene of miR-214 and negatively regulated by miR-214 [79]. In liver cancer cells, the deficiency of OGDHL upregulates αKG/citrate ratio by inhibiting of OGDHL complex activity, which in turn induces reductive TCA cycle flux and drives reductive carboxylation of glutamine-derived αKG to support lipogenesis and cellular antioxidant system, and thereby influences the chemo-sensitization effect by sorafenib [62]. Furthermore, genetic silencing of OGDHL activated mTOR signaling pathway in an αKG-dependent manner, which turned out to upregulate the expression of several key enzymes involved in lipogenesis in accompany with an increase of NADPH and glutathione levels to assist the cellular antioxidant system [62]. On the other hand, enforced expression of OGDHL increased ROS production and resulted in cell apoptosis through caspase 3-mediated suppression of AKT signaling and inhibition of NF-κB activity [76]. In pancreatic cancer cells, enhanced expression of OGDHL inhibited TWIST1 expression through ubiquitin-mediated proteolysis of HIF-1α and by regulating AKT signaling, which thereafter downregulated miR-214 expression and mitigated cell migration and invasion [79]. Three deleterious variants

(p.Val827Met, p.Pro839Leu, p.Phe836Ser) of OGDHL were predicated to associate with breast cancer risk [82]. However, in papillary thyroid cancer, the low-OGDHL patients had a better survival rate [83]. The discrepancy was attributed to different immune-related biological processes in regulating OGDHL expression, suggesting a crosstalk between OGDHL and microenvironments along tumor growth [83].

The aberrant of low-OGDHL expression was also observed in humans and in mouse models of Alzheimer's disease. A homozygous deleterious variant of OGDHL (c.C2333T; p.Ser778Leu) has been identified in patients with neurodegenerative phenotypes [61]. An increase of OGDHL expression activated Wnt/β-catenin signaling pathway and ameliorated cognitive impairment and relevant pathology in mice [84]. Wnt/β-catenin signaling pathways have been shown with protective effects on neurodegenerative diseases by maintaining the mitochondrial homeostasis [85].

DLST in cancer growth

An aberrant upregulation of DLST has been observed in several types of cancers including leukemia, triple-negative cancer cells, and neuroblastoma [86-88], which was linked to poor overall and recurrence-free survival in human triple-negative cancer patients [88]. Depletion of DLST activity suppressed cancer cell growth by provoking ROS generation, lowering NADPH production, impairing oxidative phosphorylation reaction, as well as, apoptosis induction [86-88]. Several germline variants in DLST were also observed in patients with multiple pheochromocytomas and paragangliomas and the oncogenic mechanisms were shown operating at altered TCA cycle to support cancer development [89]. Similar alterations of TCA cycle intermediated by DLST to mediate MYC-driven leukemogenesis were also observed in human T-cell acute lymphoblastic leukemia [86]. In addition, DLST can activate the pseudohypoxia pathway by interacting with mutated-EPAS1 (endothelial PAS domain-containing protein 1) [87] and promoting epigenetic modification such as DNA methylation [90].

DLDH in cancer growth

In head and neck cancer, glutaminolysis enhances cystine deprivation-induced ferroptosis via increased OGDH activity. The genetic silencing of DLDH attenuated glutaminolysis-mediated ferroptosis, in accordance with relieved lipid peroxidation, ROS, and mitochondrial iron levels [91], implicating the potential role of OGDH in ferroptosis regulation. The inactivation of DLDH by oxidative stress was restored by cysteine and glutathione replenishment [55]. The results suggested that in the genetic OGDH deficiency patients, DLDH subunit activity is defective [92].

Based on these studies, an elevation of OGDH-formed complex activity and increased circ-OGDH levels play a synergistic role in oncogenesis, while OGDHL likely functions as a tumor suppressor. However, details of the mechanisms between OGDH and OGDHL in regulation of tumor metabolism require more studies.

αKG in cancer growth

In a very early study, more glutamine consumption than other amino acids by cancer cells has been reported [93]. Glutamine is the most abundant amino acid in the circulation and glutaminolysis is executed in cancer cells more than glutamine catabolites for the production of macromolecules [68, 94]. The levels of αKG reflect the glutaminolysis activity and availability of glutamine. αKG has been shown to ameliorate autophagy by promoting mTOR signaling [61, 95] and thereby, when autophagy is inhibited, αKG accumulation from enhanced glutamine metabolism promotes proliferation of cancer cells [96] while insufficient αKG levels may suppress mitochondrial respiration and ATP generation [95]. αKG from glutamine metabolism allows OGDH to sustain NADH-associated respiration [97]. Calcium also functions in the enhancement of activities of respiratory chain components to promote oxidative phosphorylation (OXPHOS) for ATP production [98, 99]. Supplementation of αKG could rescue mitosis and viability of cancer cells due to the inhibition of Ca^{2+} transfer into mitochondria [100]. These results suggest that cancer cells greatly rely on functional TCA cycle for proliferation, in which αKG assists cancer cells to maintain a basal OXPHOS activity and adapt to the metabolic deficiencies by oxidative stress.

αKG and oncometabolites in cancer growth

Oncometabolites refer to the aberrantly accumulated metabolites, which possess pro-oncogenic capabilities to promote tumorigenesis via

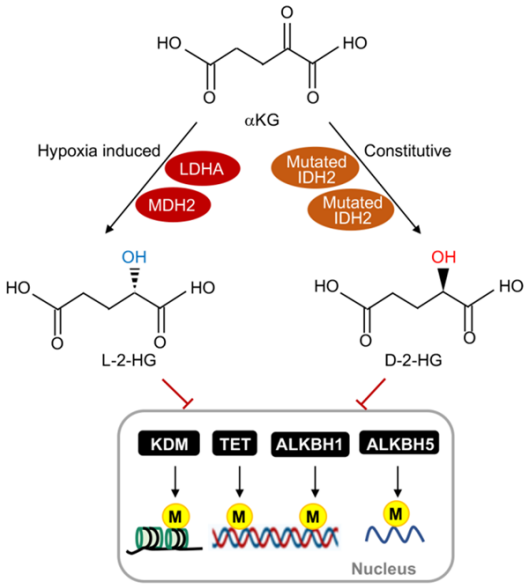

Figure 5. The role of αKG and oncometabolites in epigenetic regulation. In cancer cells, αKG can be converted to L-2-hydroxyglutarate (L-2-HG) through lactate dehydrogenase A (LDHA) and malate dehydrogenase 2 (MDH2) under hypoxia. Mutated IDH1/2 (isocitrate dehydrogenase1 and 2) can constitutively convert αKG to D-2-hydroxyglutarate (D-2-HG). Both L-2-HG and D-2-HG inhibit αKG-dependent dioxygenase activities, including histone lysine demethylases (KDMs), ten-eleven translocation proteins (TETs), and AlkB homologues (ALKBHs), which are involved in the transcriptional suppression of pro-oncogenes by maintaining the hypermethylation status of DNA and histones through epigenetic regulation.

epigenetic dysregulation, hypoxia status, and switch the phenotype of cancers [15, 101]. Oncometabolites are maintained in a limited quantity in normal tissues but persist at a higher level in cancer cells [15]. Somatic mutations of metabolic enzymes in TCA cycle including malate hydrogenase (MDH), fumarate hydratase (FH), and IDH1/IDH2 contribute to the accumulation of fumarate, succinate, L-2-HG and D-2-HG, which are generally recognized as oncometabolites [102]. Both L-2-HG and D-2-HG are derived from αKG and thus share the similar chemical structures and behaviors [102]. In normal cells, IDH converts isocitrate to αKG via a reversible oxidative decarboxylation reaction. D-2-HG is mainly generated from αKG by hydroxyacid-oxoacid transhydrogenase. However, in some cancer cells, mutations of IDH1 or IDH2 lead to the overproduction of D-2-HG [11]. Under hypoxia L-2-HG is produced by MDH or lactate dehydrogenase A (LDHA) through the reductive reaction of αKG [10, 12]. Due to the structural similarity, the two molecules behave as competitive inhibitors of αKG-dependent dioxygenases but still retain the enzyme activities, which in turn exert effects on a wide range of biological functions, leading to epigenetic dysregulation and pseudohypoxia status, and pro-oncogenic capability [13-15] (**Figure 5**). Besides, D-2-HG has been shown with inhibitory effects on OGDH in rat heart [103].

Several mutations in IDH were identified and became a significant feature in acute myeloid leukemia (AML) and gliomas [104, 105] and thus, inhibitors of mutated IDHs were developed for targeting therapy. Inhibitors of mutated IDH1 and IDH2, ivosidenib (AG-120) and enasidenib (AG-221), respectively, have been approved by FDA for IDH-mutant relapsed or refractory AML. Ivosidenib is also approved for cholangiocarcinoma and newly-diagnosed AML with a susceptible IDH1 mutation.

The family members of αKG-dependent dioxygenases include ten-eleven translocation proteins (TETs), histone lysine demethylases (KDMs), and AlkB homologues (ALKBHs). Under the inhibition of PDH activity, cellular HIF-1α is stabilized at a high level even when oxygen is abundant, termed pseudohypoxia, which can promote angiogenesis to support cancer growth [106]. Inhibition of TETs and KDMs by oncometabolites results in aberrant DNA and histone hypermethylation, respectively, and moreover affects epigenetic regulation in cancer cells [107, 108]. ALKBH serves as an N^6-mA demethylase [109, 110]. In the clinical case of SDH-mutated tumors, DNA hypermethylation and related gene silencing were observed, which reflects a result of succinate-mediated inhibition on OGDH activity [111].

Furthermore, glutamine-derived αKG has been shown to suppress M1 macrophage activation through a PHD (prolyl hydroxylase domain)-dependent post-translational downregulation of IKKβ activity, and meanwhile the polarization toward M2 macrophages is activated via engagement of fatty acid oxidation and JMJD3 (Jumonji domain-containing protein D3)-dependent epigenetic reprogramming [111]. Thereby, in this issue, elevated intracellular levels of αKG can attenuate tumor progression, suggesting that manipulations to raise cellular αKG levels may serve as a feasible strategy.

The crosstalk between OGDH and HIF-1α

An increase of cellular αKG levels can revise oncometabolite-mediated HIF-1α stabilization, in which αKG neutralizes succinate, fumarate and hypoxia-mediated activation of HIF-1α, leading to enhanced glycolysis and cell death [112, 113]. Under hypoxia and ETC defects, citrate is generated from glutamine-derived αKG through reductive carboxylation catalyzed by IDH1 and IDH2 in the cytosol and mitochondria, respectively [9, 114, 115]. In OGDH2, a splice variant of the E1 subunit of the OGDH complex, HIF-1α greatly enhanced reductive carboxylation of αKG into citrate and the enhancement was attributed to SIAH2 E3 ligase to target OGDH2 for proteasomal degradation, which switched cancer cells to require exogenous citrate or lipids for proliferation, and thereby impeded tumor growth [8]. Suppression of TCA cycle flux by HIF-1α also can be achieved by inactivating pyruvate dehydrogenase kinase 1 (PDK1) and decreased citrate levels [106, 116]. Therefore, HIF-1α intents to rewrite the metabolism by reversing TCA cycle flux from oxidation to reductive carboxylation. Inhibition of OGDH complex activity can promote reductive metabolism and induce αKG accumulation leading to reductive anabolism and L-2-HG generation, which in turn inhibits PHD activity and thus increases HIF-1α stability [24]. However, the oxidative or reductive carboxylation of αKG is reciprocally correlated depending on the citrate/αKG ratio to decide glutamine utilization. In normal conditions, glucose-derived citrate is the main source for fatty acid synthesis, while citrate shortage due to hypoxia and defects in ETC respiration can strength the reductive carboxylation of glutamine-derived αKG [66]. Enhanced reductive glutamine metabolism was observed in the cell growth of anchorage-independent tumor spheroids, which depends on the activity of cytosolic IDH1 to convert cytosolic αKG into isocitrate. Either isocitrate or isocitrate-metabolized citrate can be transported into mitochondria where they undergo oxidative carboxylation to synthesize NADPH and αKG, by which mitochondrial ROS toxicity raised by the detachment from extracellular matrix is neutralized, allowing the maximized growth of tumor spheroids [117]. Accordingly, OGDH activity is linked to HIF-α stabilization in regulating tumor growth.

The roles of OGDHc, OGDHL, and circ-OGDH in cancer cell growth are summarized in **Table 1**.

Specific targeting on OGDH for cancer therapy (Figure 6)

DLDH as therapeutic target of cancer

DLDH is a component of several complex including OGDHc, PDHc, and BCKDHc [4, 18]. With the oxidoreductive activity, DLDH is susceptible to peroxide and ROS attack but also capable of generating ROS, and thus serve as a potential anticancer target. Cancer cells often highly overexpress integrins, while DLDH can be bioengineered with Arg-Gly-Asp (RGD) motifs ($DLDH^{RGD}$), allowing to interact with integrin-rich cancer cells being internalized through integrin-associated endocytosis into cells with intact enzyme activity and ROS-production capability. Treatment of $DLDH^{RGD}$ provoked ROS production, induced cell apoptosis, and suppressed tumor growth [118]. Due to the capability of moonlighting functions, DLDH is able to adhere to multiple materials and molecules such as metal-oxides and dsDNA [119]. Treatment combining DLDH and titanium dioxide (TiO_2) was shown to elevate the selective destruction of tumor with a minimal harm to nearby normal cells [119, 120]. Whether the interaction with DLDH affects the stability of DNA and genomic function remains further investigations. However, the moonlight activity of DLDH may act on DNA alkylation for cancer treatment [120]. Due to the vulnerability of DLDH, another anticancer approach utilizing UVA to suppress DLDH expression showed ROS provocation and decreased mitochondrial membrane potential, leading to autophagic cell death and mitigated tumor growth in melanoma cells [121]. In head and neck cancer, genetic silencing of DLDH blocked cystine deprivation-induced ferroptosis [91], suggesting that DLDH mediates the cystine-regulated ferroptosis. Enhancing DLDH activity thus may serve as a tool to potentiate the anticancer effects by ferroptosis-inducing agents.

Lipoate is an important cofactor for OGDHc activity by targeting to DLDH. CPI-613 (devimistat) is a lipoate analog capable to induce E3 DLDH activity for ROS generation, leading to E2 subunit inactivation, thus impeding OGDHc activity and TCA cycle flux [122]. CPI-613 has been shown to decrease cell viability and proliferation in various types of cancers including pancreatic, breast, colon, ovarian, lung cancer and clear cell sarcoma [123-127]. Although, CPI-613 primarily targets to DLDH, other anti-

Table 1. The roles of OGDHc, OGDHL, and circ-OGDH in cancer cell growth

OGDH	Cancer growth	Mechanisms	References
OGDH	Oncogenesis (gastric cancer)	Sirt-5 inhibits cell growth and migration by inhibiting OGDH activity and hindering the succinylation of OGDH.	[57]
OGDH	Oncogenesis (gastric cancer)	OGDH enhances mitochondrial function and Wnt/β-catenin signaling	[63]
OGDH	Oncogenesis (OGDH-dependent, PI3KCA cancer cells)	OGDH suppression downregulates malate-asparate shuttle activity in OGDH-dependent and PI3KCA cancer cells.	[71, 72]
OGDH	Tumor suppressor (Hela cells)	Inhibition of OGDHc activity promotes L-2-HG, which inhibits PHDs and TETs activities.	[24]
OGDH2	Tumor suppressor (head and neck cancer cells, renal clear cell cancer cells)	Under hypoxia, OGDH2 is downregulated by HIF-1α-SIAH2. Resistant to degradation of mutated OGDH to inhibit tumor growth.	[8]
OGDHL	Tumor suppressor (pancreatic cancer cells)	Increased OGDHL inhibits TWIST1 and miR-214 via downregulation of HIF-1α, thereby impedes metastasis.	[79]
OGDHL	Tumor suppressor (pancreatic cancer cells)	MiR-193a-5p activates migration and invasion by downregulating SRSF6-OGDHL.	[80]
OGDHL	Tumor suppressor (hepatocellular carcinoma)	OGDHL suppression activates mTOR signaling, promotes lipogenesis and antioxidant capability to impair the chemosensitization to sorafenib.	[62]
OGDHL	Tumor suppressor (cervical cancer cells)	Increased OGDHL enhances caspase-3 and inhibits NF-κB.	[76]
Circ-OGDH	Oncogenesis (esophageal cancer cells)	Knockdown of circ-OGDH induces apoptosis and inhibits invasion via miR-564 downregulation- increased TPX2.	[19]
Circ-OGDH	Oncogenesis (esophageal cancer cells)	Knockdown of circ-OGDH induces apoptosis and inhibits invasion via miR-615-5p downregulation- increased PDX1.	[20]
DLST	Oncogenesis (T-cell acute lymphoblastic leukemia)	DLST mediates MYC-driven leukemogenesis.	[86]
DLST	Oncogenesis (neuroblastoma, pheochromocytoma-paraganglioma)	DLST activates pseudohypoxia pathway by interacting with mutated-EPAS1 and promoting DNA methylation.	[87, 90]
DLST	Oncogenesis (pheochromocytoma-paraganglioma)	DLST alters TCA cycle to support cancer growth.	[89]

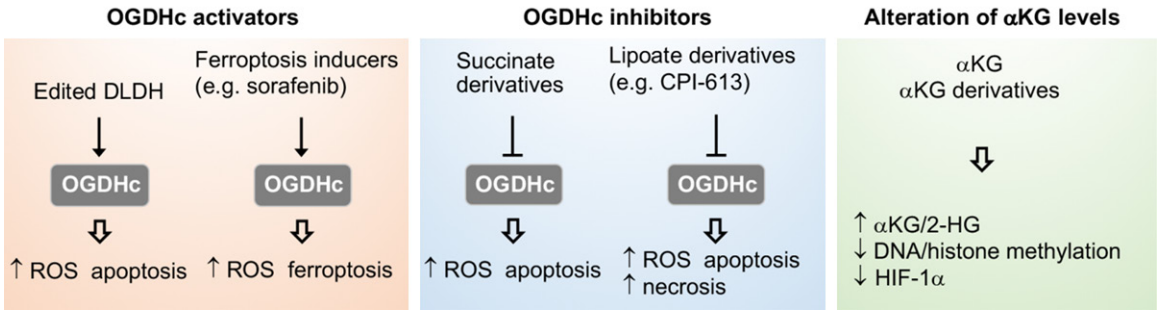

Figure 6. OGDHs serves as a target for cancer treatment. Engineered DLDH or ferroptosis inducers such as sorafenib acting on xCT can activate OGDH to promote ROS production and cancer cell death. Derivatives of succinate and lipoate induce cancer cell death via OGDH inhibition-mediated ROS provocation. Supplementation with exogenous αKG or αKG derivatives attenuates cancer cell growth by elevating αKG/2-hydroxyglutarate (2-HG) ratio followed by inhibiting DNA/histone methylation and/or HIF-1α stability.

cancer mechanisms were also discovered. For example, CPI-613 enhances pancreatic apoptosis through AMPK-ACC signaling to rewires lipid metabolism [126]. In clear cell sarcoma, CPI-613 induces autophagosome formation followed by lysosome fusion, leading to cell necrosis [124]. CPI-613 is currently evaluated in clinical studies as a single agent or in combination with standard drug therapies for patients with pancreatic cancer (NCT03699319), T-cell non-Hodgkin lymphoma (NCT04217317), clear cell sarcoma (NCT04593758), Burkitt's lymphoma (NCT03793140), and biliary trace cancer (NCT04203160).

Derivatives of succinate

Succinyl phosphonate and glutaryl phosphonate analogs of succinate have been used to blunt OGDH activity and tested for their anticancer capability in laboratory studies [57, 70, 107, 128, 129]. Another OGDH inhibitor, a

derivative of succinate (S)-2-[(2,6-dichlorobenzoyl) amino] succinic acid (AA6), is also able to inhibit tumor growth by raising cellular αKG levels in an orthotopic mouse model of breast cancer 4T1 and human breast cancer cells [130].

Alterations of intracellular αKG levels

αKG is involved in a wide range of cellular processes in both physiological and pathological conditions. In cancer cells, αKG protects cells from oxidative insults, modulates protein and lipid synthesis, and participates in the β-oxidation of fatty acids. αKG can serve as a substrate of αKG-dependent dioxygenases to manipulate epigenetic modifications. Besides, αKG suppresses HIF-1α stability [24]. Due to the critical role of αKG in cancer cell metabolism and transcription programming, its metabolites as anticancer agents to modulate oncogenic process have been widely evaluated. An increase of αKG has been reported to reverse the inhibition of 2-HG on histone demethylase [15]. Accumulation of αKG by OGDH inhibition promoted TET1, TET3 protein expression, and enzyme activity in breast cancer, and impeded cell migration and epithelial-mesenchymal transition [130]. Furthermore, defective Complex I was shown to increase cellular αKG levels to destabilize HIF-1α under hypoxia, leading to suppress tumor growth in both *in vitro* and *in vivo* studies [131, 132]. Under hypoxia conditions, treatment of exogenous αKG inhibited HIF-1α stability in a dose-dependent manner [133]. Exogenous αKG also suppressed tumor growth and ameliorated metastasis of breast cancer cells by switching metabolism from glycolytic and oxidative status [134]. Based on these findings, exogenous supplementation of αKG to raise its intracellular levels is proposed as a possible anticancer strategy. Due to low cell permeability, ester derivatives of αKG were developed to increase the permeability and availability within cells [112, 113]. Treatment with these derivatives reversed pseudohypoxia induced by the loss of SDH or fumarate hydratase and prevented hypoxia-mediated invasion by reactivating PDH activity to impair HIF-1α stabilization [112, 113]. Furthermore, supplementation of these derivatives in cancer cells significantly modulated HIF-1α-mediated effects such as angiogenesis and metabolic alterations, and more importantly induced cancer cell apoptosis [112, 113, 133, 135, 136]. Treatment with αKG was further shown to induce histone demethylation via JMJD-mediated senescence [137]. The supplementation of dimethyl-αKG could kill cancer cells when combined with anticancer agent or inhibitors of OXPHOS [138]. Collectively, αKG indeed exerts a positive anticancer effect on different cancer types. Since αKG is a native metabolic intermediate with a low toxicity to normal cells, αKG apparently serves as a potential candidate in pharmacological development for cancer treatment. However, more extensive investigations are required to evaluate the efficacy of αKG for clinical therapy. It has been demonstrated that αKG becomes a culprit to activate the αKG-dependent dioxygenase family enzymes and drive oncogenic transformation via hypoxia adapting and shaping of the epigenetic landscape [139].

Conclusions

In this review, we highlight OGDH as a key role to potentiate cancer cells against crucial alterations of the environments. With ROS production and antioxidative function, OGDH activity governs OXPHOS oscillations, manipulates the mitochondrial redox status through ROS and NADH generation, and guides the metabolite flux in TCA cycle towards energetic or anabolic process, as well as the corresponding signaling pathways. The consequent signaling pathways by altered OGDH activity may be amplified and extended outside of mitochondrion, which drives the systemic remodeling of cells. Next to glucose, glutamine works as a major energy source for cancer cells. Cancer cells rely on high levels of exogenous glutamine due to the catabolism into αKG as a fuel to replenish TCA cycle. αKG operates at the intersection linking TCA cycle and glutamine metabolism, coordinating the energy production, redox regulation, and macromolecule synthesis. αKG also functions as the substrate or co-factor to regulate biological processes. Dysfunctional αKG metabolism by hypoxia and mutated IDH enzymes promotes the generation of oncometabolites, which in turn can change epigenetic landscape and shape cell metabolism, allowing the adaption of cancer cells. In line with this, OGDH and αKG serve a hub in cancer growth and proliferation. Small molecules specifically targeting OGHD have been demonstrated with pronounced effects and efficacy against cancers

in animal models and clinical trials. The key cancer metabolites have been proved as potential targets for anti-tumor therapies. However, since cancer cells may undergo a variety of regulatory loops to compensate the disturbed pathways and drought of metabolites, most single target therapies tend to fail. Therapies in combination with multiple targets, and more comprehensive studies are needed to improve the therapeutic effects.

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Disclosure of conflict of interest

None.

Abbreviations

2-HG, 2-hydroxyglutarate; ALKBH, AlkB homologue; αKG, α-ketoglutarate; BDKDHc, branched-chain ketoacid dehydrogenase complex; CoA, Coenzyme A; DLDH, dihydrolipoamide dehydrogenase; D-2-HG, D-2-hydroxyglutarate; DLST, dihydrolipoamine succinyltransferase; ETC, electron transport chain; FH, fumarate hydratase; GLS, glutaminase; GLUD, glutamate dehydrogenase; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase; Grx2, glutathione reductase 2; GS, glutamine synthetase; HIF-1α, hypoxia inducible factor-1α; HNE, 4-hydroxy-2-nonenal; IDH, isocitrate dehydrogenase; KAT2A, lysine acetyltransferase 2; KDM, lysine demethylases; KGDH, α-ketoglutarate dehydrogenase; L-2-HG, L-2-hydroxyglutarate; LDHA, lactate dehydrogenase A; mTOR, mammalian target of rapamycin 1; OGDHc, oxoglutarate dehydrogenase complex; OGDHL, OGDH-like; OXPHOS, oxidative phosphorylation; PDHc, pyruvate dehydrogenase complex; PHDs, prolyl hydroxylases; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SCS, succinyl-CoA synthetase; TCA, tricarboxylic acid; TET, ten-eleven translocation hydroxylase; TPP, thiamine pyrophosphate.

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