

REVIEW

Open Access



# The Warburg effect modulates DHODH role in ferroptosis: a review

Alvan Amos<sup>1,2</sup>, Alex Amos<sup>3</sup>, Lirong Wu<sup>1</sup> and He Xia<sup>1\*</sup>

## Abstract

Ferroptosis is an iron-dependent regulated cell death that suppresses tumor growth. It is activated by extensive peroxidation of membrane phospholipids caused by oxidative stress. GPX4, an antioxidant enzyme, reduces these peroxidized membrane phospholipids thereby inhibiting ferroptosis. This enzyme has two distinct subcellular localization; the cytosol and mitochondria. Dihydroorotate dehydrogenase (DHODH) complements mitochondrial GPX4 in reducing peroxidized membrane phospholipids. It is the rate-limiting enzyme in de novo pyrimidine nucleotide biosynthesis. Its role in ferroptosis inhibition suggests that DHODH inhibitors could have two complementary mechanisms of action against tumors; inhibiting de novo pyrimidine nucleotide biosynthesis and enhancing ferroptosis. However, the link between mitochondrial function and ferroptosis, and the involvement of DHODH in the ETC suggests that its role in ferroptosis could be modulated by the Warburg effect. Therefore, we reviewed relevant literature to get an insight into the possible effect of this metabolic reprogramming on the role of DHODH in ferroptosis. Furthermore, an emerging link between DHODH and cellular GSH pool has also been highlighted. These insights could contribute to the rational design of ferroptosis-based anticancer drugs.

**Keywords** Mitochondria, Ferroptosis, Dihydroorotate dehydrogenase, Ribonucleotide reductase, Electron transport chain, Superoxide anion

## Background

Oncogenic mutations significantly contribute to tumorigenesis. An example is the mutation that affects Ras superfamily of small GTPases [1]. It was discovered that two chemical compounds; erastin and Ras-selective lethal compound 3 (RSL3) selectively induce cell death in Ras-mutant cancers. This cell death mechanism lacks the classical molecular and biochemical features of

apoptosis. Rather, it was characterized by a high cellular level of oxidative stress markers and also susceptible to inhibition by iron chelation or blocking cellular iron uptake [2]. To capture its iron dependency, this novel cell death mechanism was termed “ferroptosis” [2]. RSL3 inhibits glutathione peroxidase 4 (GPX4), an antioxidant enzyme having two distinct subcellular localization; the cytosol and the mitochondria [3]. This enzyme protects membrane phospholipids from peroxidation [4]. Erastin is an inhibitor of cystine-glutamate antiporter (xCT), a transmembrane protein that imports cystine into the cell [5]. Cystine is then reduced to cysteine, the rate-limiting amino acid in the biosynthesis of reduced glutathione (GSH) [5]. GSH serves as a cofactor for GPX4.

Following its first description, ferroptosis has been implicated in the pathogenesis of cancer, neurological disorders, coronary heart diseases, liver, and kidney diseases [6]. However, most of the studies focused on the involvement of ferroptosis in cancer, and a recent

\*Correspondence:

He Xia  
hexiabm@163.com

<sup>1</sup> Department of Radiation Oncology, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, 42 Baiziting Road, Nanjing 210009, China

<sup>2</sup> Department of Biochemistry, Faculty of Science, Kaduna State University, PMB 2339 Tafawa Balewa Way, Kaduna, Nigeria

<sup>3</sup> Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Zaria, Nigeria



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

finding is the contribution of dihydroorotate dehydrogenase (DHODH) to ferroptosis inhibition. It was reported that DHODH works along with mitochondrial GPX4 to inhibit ferroptosis [3]. DHODH is the rate-limiting enzyme in de novo pyrimidine nucleotide biosynthesis located in the mitochondria. It links de novo pyrimidine nucleotide biosynthesis to the electron transport chain (ETC) at complex III through Coenzyme Q (ubiquinone) pool [7]. This suggests that in addition to suppressing tumor growth by inhibiting pyrimidine biosynthesis, DHODH inhibitors could have a second mechanism of action that is, enhancement of ferroptosis.

In contrast to normal cells, which rely on mitochondrial oxidative phosphorylation to generate adenosine triphosphate (ATP), most cancer cells instead use aerobic glycolysis also known as the Warburg effect [8]. This metabolic reprogramming could be a biochemical adaptation that supports the biosynthetic requirements of rapidly proliferating cells [9]. Since nucleic acid synthesis is a key biosynthetic requirement of cancer cells, the metabolic reprogramming associated with the Warburg effect could enhance its efficiency. It is important to note that the Warburg effect affects the functioning of mitochondrial ETC which is linked to the de novo pyrimidine nucleotide biosynthesis by DHODH at complex III [7]. Given the role of the ETC in the generation of oxidative stress-inducing reactive oxygen species (ROS) and the involvement of DHODH in ETC function; we reviewed previous studies to understand the possible effect of the Warburg effect on the role of DHODH in ferroptosis.

### The electron transport chain and ferroptosis

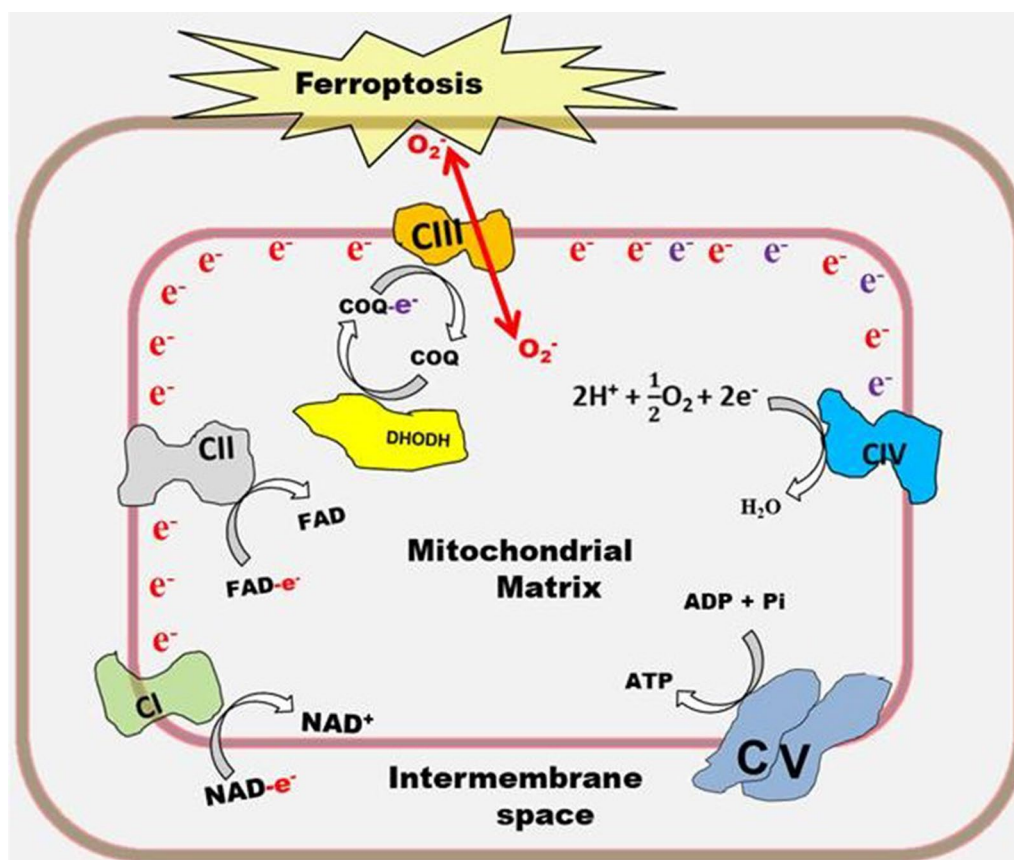
The ETC is a series of inner mitochondrial membrane protein complexes and biochemical mobile electron carriers which facilitate the movement of electrons extracted from fuel molecules to oxygen to form water [10]. Electron movement along the ETC occurs with a concurrent translocation of protons into the intermembrane space to generate a proton gradient. The proton gradient is dissipated when  $H^+$  re-enters the mitochondrial matrix through ATP synthase also known as complex V [11]. The other protein complexes are complex I, complex II, complex III, and complex IV. Electron leakage along the ETC produces  $O_2^-$ . This primarily occurs at complex I or complex III [12].

It was reported that  $O_2^-$  produced by complex III of the ETC plays a pivotal role in the induction of ferroptosis by cysteine deprivation [13]. The study revealed that S3QEL, a molecule that suppresses  $O_2^-$  production by complex III and not S1QEL which suppresses  $O_2^-$  generation by complex I could protect mouse hepatoma Hepa 1–6 cells from lipid peroxidation and ferroptosis. In addition, S3QEL was able to inhibit ferroptosis in xCT-knockout

mouse-derived embryonic fibroblasts [13]. The xCT knock out (KO) causes cysteine deficiency which inhibits glutathione biosynthesis. This study suggests that the functionality of complex III of the ETC may be required for pharmacological induction of ferroptosis. The ability of complex III-generated  $O_2^-$  to induce lipid peroxidation could be due to its bi-directional release both into the mitochondrial matrix and the intermembrane space (Fig. 1) as opposed to complex I-generated  $O_2^-$  which is only released into the mitochondrial matrix [12].

It was earlier reported that the biochemical mechanism for the induction of ferroptosis by erastin is mitochondrial membrane potential hyperpolarization [14]. However, this study did not report the dynamics of  $O_2^-$  generation by the ETC under experimental conditions. It is conceivable that the membrane potential hyperpolarization observed during the study was linked to ferroptosis induction due to a positive correlation with  $O_2^-$  generation by the ETC. This view is supported by the failure of complex I and II inhibitors to sustainably suppress ferroptosis during the study, suggesting that provided electrons pass through complex III and IV, ferroptosis could be activated. The generation of  $O_2^-$  by complex III could be a more plausible hypothesis explaining the biochemical mechanism underlying ferroptosis induction by erastin. This is because of the well-established relationship between ferroptosis and oxidative stress.

A third study that reported the biochemical mechanism underlying the induction of ferroptosis by erastin also implicated  $O_2^-$  generation [15]. However, this study concluded that the ETC is not involved in erastin-induced ferroptosis but rather, the pyruvate dehydrogenase complex-catalyzed reaction. This is because treating HT1080 cells with Antimycin A or FCCP was found to have no significant effect on the cell's sensitivity to erastin as opposed to the genetic inhibition of E1 or E3 subunits of pyruvate dehydrogenase complex. This study used changes in the  $E_{50}$  value of erastin as indicative of ferroptosis sensitivity. A possible drawback to the validity of the conclusion dissociating ETC from erastin-induced ferroptosis is the absence of data showing that the cells actually died as a result of ferroptosis. This data may be important because Antimycin and FCCP can interact with erastin to modify its pharmacological effect. In addition, although the decrease in oxygen consumption rate (OCR) observed as a result of Antimycin treatment is expected to correlate with decreased electron flow through the ETC resulting in decreased  $O_2^-$  production, a previous study has reported an increase in cellular  $O_2^-$  level in response to Antimycin treatment [16]. If this happens to be the case in this study, although a significant difference in OCR was observed between the Antimycin



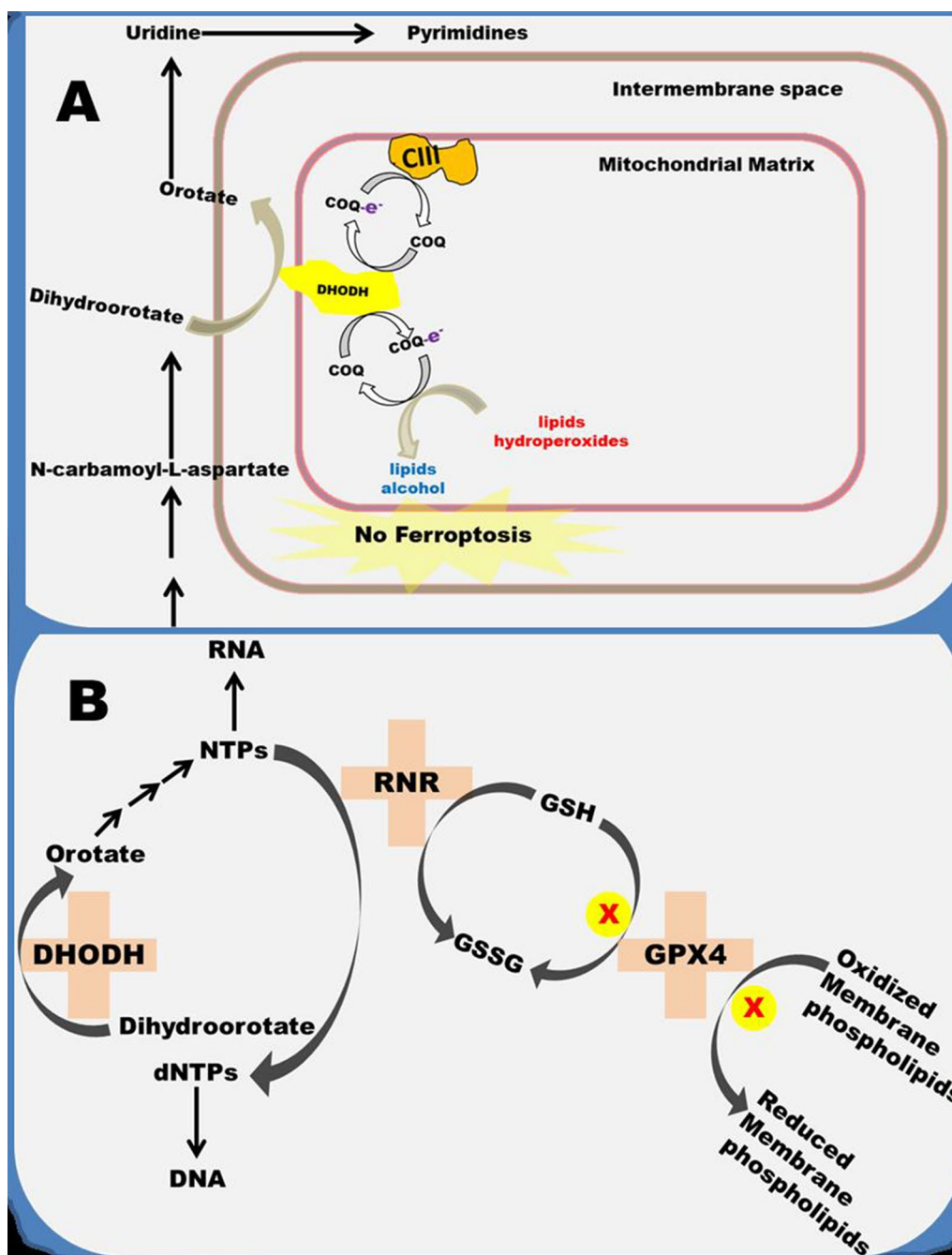
**Fig. 1** Contribution of ETC to ferroptosis induction. Electrons shuttled into the ETC come from NADH (represented as  $\text{NAD-e}^-$ ),  $\text{FADH}_2$  (represented as  $\text{FAD-e}^-$ ), and  $\text{CoQ}_{10}\text{H}_2$  (represented as  $\text{COQ-e}^-$ ). Complex III (CIII)-generated  $\text{O}_2^{\cdot-}$  has been shown to play a key role in the induction of ferroptosis activated by cysteine deprivation

and FCCP treated group, there may be no significant difference in cellular  $\text{O}_2^-$  levels. Therefore, the use of a different pharmacological agent like S3QEL or a genetic approach to the inhibition of complex III of the ETC may tell a different story.

#### DHODH and ferroptosis

DHODH functions along with mitochondrial GPX4 to reduce peroxidized membrane phospholipids [17]. Metabolomics screening following the treatment of cancer cells with GPX4 inhibitors resulted in a significant depletion of *N*-carbamoyl-L-aspartate and the accumulation of uridine. *N*-carbamoyl-L-aspartate is a de novo pyrimidine nucleotide biosynthesis intermediate located upstream of DHODH while uridine is an intermediate downstream of DHODH. These changes suggest that GPX4 inhibition enhanced the conversion of *N*-carbamoyl-L-aspartate to uridine. Given that DHODH is the rate-limiting enzyme of this pathway, it is conceivable that the flow of metabolites can only increase if DHODH activity is upregulated. Supplementing the culture

medium with dihydroorotate, a substrate for DHODH, suppressed ferroptosis activated by GPX4 inhibition while supplementing the medium with uridine, the end product of the pathway, enhanced ferroptosis induced by GPX4 inhibition. These observations suggested a complementary role between GPX4 and DHODH in ferroptosis inhibition, a hypothesis that was later confirmed during the study [17]. Further investigations revealed that the synergy is between mitochondrial GPX4 and DHODH. A mechanistic study showed that the lipophilic antioxidant, ubiquinol ( $\text{CoQ}_{10}\text{H}_2$ ), produced during DHODH-catalyzed conversion of dihydroorotate to orotate is the anti-ferroptosis molecule. This mitochondria-localized ferroptosis defense mechanism has its cytosolic counterpart involving the conversion of ubiquinone ( $\text{CoQ}_{10}$ ) to  $\text{CoQ}_{10}\text{H}_2$  by ferroptosis suppressor protein 1 (FSP-1) [18]. Whether other ubiquinol-generating reactions within the mitochondria inhibit ferroptosis is yet to be investigated. This biochemical mechanism linking DHODH with ferroptosis is represented in Fig. 2A.



**Fig. 2** **A** Ferroptosis inhibition by DHODH. DHODH-generated Ubiquinol (COQ-e-) reduces mitochondrial membrane phospholipids hydroperoxides to lipids alcohol, thereby inhibiting the onset of ferroptosis. **B** Ferroptosis induction by DHODH. DHODH-catalyzed reaction indirectly reduces the cellular level of GSH. This is because the nucleoside triphosphates (NTPs) it produces are required for RNR-catalyzed reaction which uses GSH as a cofactor. This can inhibit GPX4 via GSH depletion

A second link between DHODH and ferroptosis is found within the deoxyribonucleic acid (DNA) biosynthetic pathway. Nucleosides triphosphates (NTPs), which are the precursors for deoxynucleosides triphosphates (dNTPs) used for DNA synthesis, are derived from

purines and pyrimidines [19]. Given that during DNA synthesis, purines pair with pyrimidines, insufficient pyrimidines could terminate the base-pairing process. The de novo pyrimidine biosynthetic pathway plays an important role in rapidly proliferating cells. This is due



to the need to meet the cell's high nucleic acid demand. Since DHODH catalyzes the rate-limiting step in de novo pyrimidine biosynthesis, its inhibition could decrease the cellular levels of pyrimidines. Low cellular levels of pyrimidines will slow down ribonucleotide reductase (RNR)-catalyzed reaction which converts NTPs to dNTPs. This assertion is supported by a significant decrease in dNTPs observed following DHODH inhibition [20]. A recent study reported that ferroptosis could be suppressed by inhibiting RNR [21]. This is because RNR uses GSH as a cofactor. When RNR is inhibited, the cellular GSH pool increases. This becomes available to GPX4 to use for reducing peroxidized membrane phospholipids. Therefore, DHODH depletion could enhance the efficiency of GPX4 via inhibiting RNR. However, DHODH depletion may not inhibit ferroptosis in cells with low GPX4 expression via this biochemical mechanism. This biochemical mechanism linking DHODH with ferroptosis is represented in Fig. 2B.

#### The role of DHODH and complex III in tumor growth

Tumor cells are characterized by rapid growth and division. As a result, most of the biosynthetic precursors are synthesized de novo [22]. To sustain growth in tumors, cell division must progress rapidly. This cannot occur when biosynthetic precursors required for the S phase of the cell cycle are insufficient. The S phase facilitates chromosome doubling that precedes cell division. This phase in tumors will require a large supply of pyrimidines that can only be made available when synthesized de novo. DHODH, the rate-limiting enzyme for de novo pyrimidine nucleotide biosynthesis, links this pathway to complex III of the ETC [23]. The reaction mechanism for DHODH involves the reduction of ubiquinone (CoQ10) to CoQ10H<sub>2</sub>. CoQ10 is a mobile electron carrier that accepts electrons at complexes I and II of the ETC and transfers them to complex III. However, DHODH-generated CoQ10H<sub>2</sub> donates its electrons directly to complex III [23]. Therefore, inhibition of complex III will not only halt electron flow through the ETC but could also inhibit DHODH-catalyzed conversion of dihydroorotate to orotate, thereby shutting down de novo pyrimidine nucleotide biosynthesis. This could result in cell cycle arrest and the inhibition of tumor growth. Inhibition of complex III of mitochondrial ETC by antimycin A was able to inhibit proliferation and promote cellular differentiation in acute myeloid leukemia (AML) cells through a DHODH inhibition-mediated mechanism [24].

Another study reported impaired tumor growth due to a dysfunctional complex III of the ETC. The biochemical mechanism underlying this growth inhibition involves the failure of complex III to re-oxidize CoQ10H<sub>2</sub> back to CoQ10 rather than the failure of electrons to flow

through the ETC [25]. During the study, ectopic expression of *Ciona intestinalis* alternative oxidase (AOX) which oxidizes CoQ10H<sub>2</sub> to CoQ10 restored the lost tumor growth. In addition, a functional complex III and IV of the ETC alone sufficiently restored tumor growth of respiration-deficient cancer cells through the re-activation of de novo pyrimidine nucleotide biosynthesis [26]. These studies provide mechanistic insight into a previous observation where the acquisition of mitochondrial DNA (mtDNA) via horizontal transfer of whole mitochondria from host cells to mtDNA-deficient cancer cells restored lost tumorigenicity [27, 28]. The transferred host mitochondria could have facilitated the rate-limiting step in de novo pyrimidine nucleotide biosynthesis. Also, these findings are an addition to the accumulating evidence against complete mitochondrial damage as the biochemical basis for aerobic glycolysis in tumors. During aerobic glycolysis also known as the Warburg effect, some aspects of mitochondrial function could be inefficient; however, the ETC-linked DHODH-catalyzed rate-limiting step for de novo pyrimidine nucleotide biosynthesis should not be affected. This conclusion is further supported by the need for a functional complex III for hepatitis E virus replication [29]. In fact, disruption in certain aspects of mitochondrial function during aerobic glycolysis, in addition to conferring other tumorigenic advantages, appears to be aimed at optimizing de novo pyrimidine nucleotide biosynthesis. This optimization could be due to the high nucleic acid demand during the S-phase of the cell cycle [30]. For example, hypoxia can slow down the movement of electrons through the ETC due to insufficient oxygen molecules that can serve as terminal electron acceptors. A defect in complex I and II of the ETC associated with the Warburg effect [31, 32] could enable more efficient coupling of mitochondrial oxygen consumption to the conversion of dihydroorotate to orotate in an oxygen-deficient intracellular environment predominant in solid tumors.

The importance of de novo pyrimidine nucleotide biosynthesis for the proliferation of human Jurkat leukemic T cells is indirectly substantiated by a metabolic reprogramming activated by complex I inhibition. When the ETC is fully functional, glutamate-oxaloacetate transaminase 1 (GOT1) converts aspartate to oxaloacetate and  $\alpha$ -ketoglutarate to glutamate concurrently. This reaction is part of the cytosolic portion of the malate-aspartate shuttle that transfers NADH produced during glycolysis from the cytosol into the mitochondria. The mitochondrial matrix portion of this shuttle comprises malate dehydrogenase 2 (MDH2) and glutamate-oxaloacetate transaminase 2 (GOT2). These enzymes synthesize aspartate which is transported to the cytosol for use by GOT1 and other enzymes. However, complex I inhibition

inhibits the regeneration of  $\text{NAD}^+$  by the ETC. This leads to the inhibition of the mitochondrial matrix portion of the shuttle. Under this condition, GOT1-catalyzed reaction reverses, thereby producing aspartate from the oxaloacetate produced by ATP citrate lyase-catalyzed reaction in the cytosol. This citrate comes from the reductive decarboxylation of glutamine in the mitochondrial matrix [33]. Following complex I inhibition, these cells were found to rely on GOT1-dependent aspartate biosynthesis for proliferation [33].

A defect in complex I of the ETC rewires the malate-aspartate shuttle, shutting down GOT2-dependent aspartate biosynthesis while activating GOT1-dependent aspartate biosynthesis which is linked to the reductive decarboxylation of glutamine. This could be the reason why tumors which usually have a defective ETC due to the Warburg effect are heavily reliant on glutamine metabolism for proliferation [34, 35]. It is important to note that during the study, experimental data suggested that a significant fraction of the aspartate synthesized by GOT1 goes to purine and pyrimidine nucleotide biosynthesis [22]. Given that, GOT1-dependent aspartate biosynthesis could only support cell proliferation if DHODH and complex III are functional. The coupling of DHODH-catalyzed reaction to complex III of the ETC [36] suggests that reductive decarboxylation of glutamine which supplies the oxaloacetate used by GOT1 could result in the generation of ATP in the ETC through DHODH-mediated shuttling of  $\text{CoQ10H}_2$  to complex III [37].

High extracellular lactate characteristic of the Warburg effect induces reductive decarboxylation of glutamine in breast cancer cells [38] and tumor hypoxia which can also induce the Warburg effect making aspartate become a limiting metabolite for cell proliferation [39]. Aspartate was found to be limiting for cell proliferation because of its role in de novo purine and pyrimidine nucleotide biosynthesis. In pyrimidine biosynthesis, aspartate contributes three carbon atoms during the production of uridine monophosphate (UMP) and thymidine monophosphate (TMP) [39]. Under hypoxia and ETC dysfunction, 50 to 80% of orotate, dTMP, and UMP in solute carrier family 1 member 3 (SLC1A3)-expressing cells were derived from extracellularly imported aspartate. This suggests that hypoxia could reduce the intracellular concentration of pyrimidines synthesized de novo by more than 50% unless an adaptive metabolic rewiring occurs to supply aspartate. This study suggests a relationship between defective complex I, GOT1-facilitated aspartate biosynthesis, and nucleotide biosynthesis.

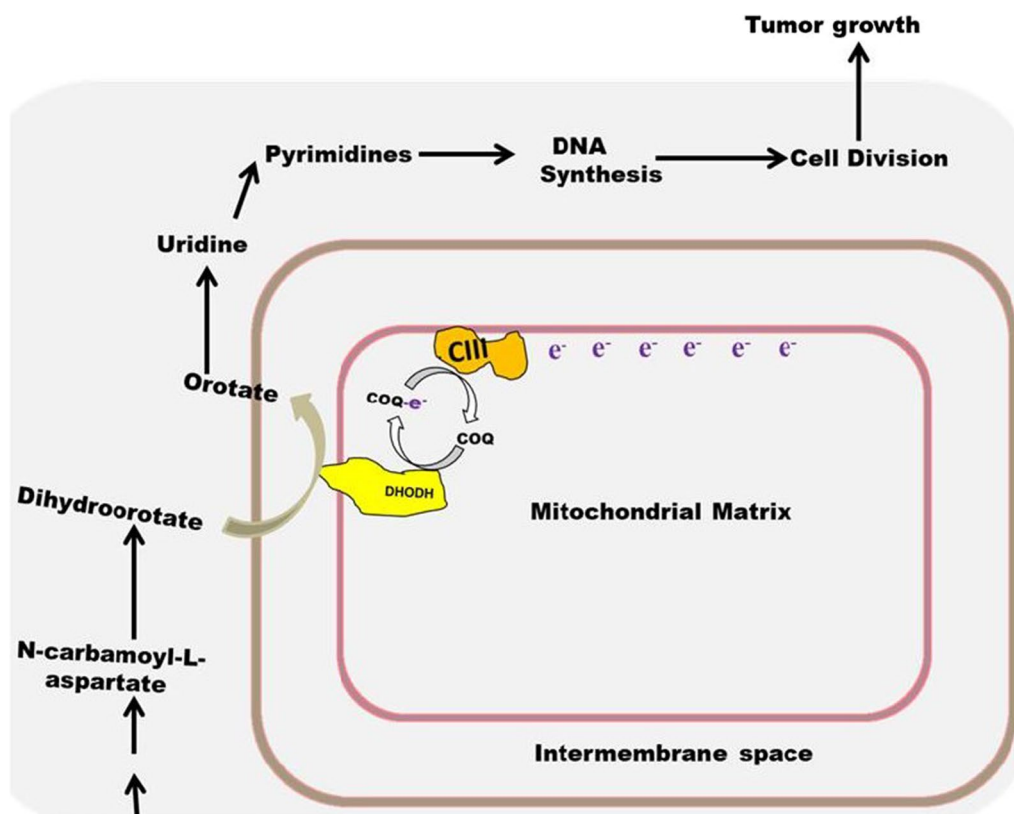
Coupling of de novo pyrimidine nucleotide biosynthesis to the ETC could be a strategy for meeting the high energy demand for pyrimidine biosynthesis when metabolic rewiring occurs that shuts down part of the

energy-generating mitochondrial catabolism in favor of energy-demanding nucleotide biosynthesis. This is because DHODH-facilitated electron transfer to complex III and IV could result in pumping protons into the intermembrane space thereby generating some transmembrane electrochemical potential that could be used for ATP synthesis by complex V [40]. This notion is supported by the efficient induction of mitochondrial membrane potential depolarization by DHODH inhibitors from two distinct chemical series [41]. Findings from another study suggest that preceding UVB-Induced primary skin tumor formation, a metabolic rewiring occurs that shuts down glycolysis and the TCA cycle (thereby shutting down complex I and II) while upregulating the distal part of the ETC which includes a heightened DHODH activity [42]. In this study, DHODH was found to sustain ATP generation by the ETC.

Other defects in mitochondrial function that could selectively shut down complex I and II while preserving DHODH-catalyzed reaction and complex III include a tumor-associated defect in three enzymes of the TCA cycle [43]. These enzymes are succinate dehydrogenase (SDH), fumarate hydratase (FH), and isocitrate dehydrogenase (IDH). Defects in these enzymes affect cellular ROS status in a way that induces oxidative stress and stabilizes HIF1- $\alpha$ . While a deficiency in SDH increases ROS levels that result in HIF-1 $\alpha$  stabilization, IDH1 and IDH2 mutation lowers the level of GSH and NADPH thereby reducing the efficiency of GSH and NADPH-dependent antioxidant enzymes. However, to manifest the metabolic phenotype of SDH mutant tumors, additional inhibition of complex I is required [44]. The absence of complex I inhibition has been postulated to be the differentiating factor between neurodegeneration-linked SDH deficiency and tumor-linked SDH deficiency [44]. A functional complex I could support glutaminolysis and the malate-aspartate shuttle, two pathways that could transfer electrons to complex I thereby reducing the efficient coupling of DHODH-catalyzed reaction to the utilization of the limited oxygen available in a tumor hypoxic environment. The mutation in FH inhibits complex I through the succination of its Fe-S proteins and complex II through a feedback inhibition activated by fumarate [45]. Role of DHODH and complex III in tumor growth is schematically represented in Fig. 3.

#### **Warburg effect and its modulation of the role of DHODH in ferroptosis**

Although DHODH complements mitochondrial GPX4 to inhibit ferroptosis, the prevalence of Warburg effect in tumors, indispensability of DHODH-catalyzed reaction for tumor growth and the role of ETC in  $\text{O}_2^-$  generation and ferroptosis induction suggest that inhibiting



**Fig. 3** Role of DHODH and complex III in tumor growth. DHODH-catalyzed reaction is the rate-limiting step in de novo pyrimidine nucleotide biosynthesis. It catalyzes the conversion of dihydroorotate to orotate used for nucleic acid biosynthesis. This reaction needs the activity of complex III of the ETC and it involves the conversion of ubiquinol (COQ-e<sup>-</sup>) to ubiquinone (COQ)

DHODH in some tumors could result in ferroptosis inhibition. This could result in drug resistance. Solid tumors are characterized by oxidative stress [46]. As ROS inhibit complex I and II of the ETC and the TCA cycle, it stabilizes HIF1- $\alpha$  which upregulates glycolysis as an adaptive response for ATP generation [47]. HIF1- $\alpha$  is an important component of HIF-1, a transcription factor that targets key glycolytic enzymes [47]. Active HIF-1 complex is a heterodimer made up of an oxygen-sensitive HIF-1 $\alpha$  subunit and a constitutively expressed HIF-1 $\beta$  subunit. The HIF-1 $\alpha$  subunit has an oxygen-dependent degradation domain (ODD) which contains two regulatory prolyl residues; P402 and P564. Under normal cellular oxygen concentration, these amino acid residues become hydroxylated by HIF prolyl-hydroxylases (PHDs). HIF-1 prolyl-hydroxylation facilitates the proteasomal degradation of this transcription factor [48]. However, even in the presence of oxygen, studies have shown that HIF-1 $\alpha$  can be stabilized by ROS produced by the ETC.

In a study to establish the molecular mechanism underlying the activation of HIF-1 by angiotensin-II in vascular smooth muscle cells, it was discovered that ROS produced by the ETC play a key role [49]. The use of

mitochondrial ETC complex III inhibitors, stigmatellin, and myxothiazol were found to inhibit the accumulation of HIF-1 induced by angiotensin-II. A second approach used in the study involved suppressing ROS production through siRNA-mediated inhibition of Rieske Fe-S protein, an essential component of complex III. This equally resulted in the inhibition of HIF-1 $\alpha$  accumulation. The use of SkQ1, an antioxidant targeting mitochondrial ETC also produced the same result. This suggests that O<sub>2</sub><sup>-</sup> generated by the ETC plays an important role in the stabilization of HIF-1 $\alpha$ . Genetic and pharmacological inhibition of NADPH oxidase (NOX), a mitochondrial enzyme that produces O<sub>2</sub><sup>-</sup> failed to sustain HIF-1 $\alpha$  accumulation under non-hypoxic conditions [49]. This study concluded that ROS generated by the mitochondria is responsible for blocking HIF-1 $\alpha$  hydroxylation and its subsequent proteasomal degradation during Angiotensin II treatment in vascular smooth muscle cells. Stabilization of HIF-1 by oxidative stress induces the Warburg effect.

Another study using hepatoma cells also supports the involvement of oxidative stress in inducing the Warburg effect even in the presence of oxygen [50]. During

this study, it was discovered that the ability of hypoxia to upregulate glycolysis is dependent on ROS. This is because, in the presence of ROS scavengers, hypoxia failed to upregulate glycolysis. Hypoxia could increase both ROS level and HIF1- $\alpha$  activity. However, when ROS level was reduced using the antioxidant  $\alpha$ -Lipoic Acid, the expression of HIF1- $\alpha$  was downregulated. The study further revealed that increasing endogenous ROS generation by xanthine oxidase gene transfection or decreasing it using antioxidants or MnSOD transfection could modulate cellular glycolytic activity independent of hypoxia status. Another group used mitoubiquinone, a mitochondria-targeted antioxidant to confirm the involvement of mitochondrial ROS in the stabilization of HIF-1 $\alpha$  [51]. Furthermore, data from a study designed to assess the limiting metabolites required for cell proliferation under hypoxia suggests that tumor hypoxia is sufficient to inhibit the ETC [39]. It should be noted that if this inhibition affects complex III, tumor growth could be suppressed.

PTEN-induced kinase 1 (PINK1) inhibits the Warburg effect by reducing mitochondrial ROS generation. It also suppresses the growth of glioblastoma [52] suggesting that the Warburg effect, which is characterized by defective ETC, supports tumor growth. PINK1 suppresses ROS and tumor growth through FOXO3a, a transcription factor that regulates SOD2. ROS-mediated stabilization of HIF1- $\alpha$  was observed in PINK1 knockout (KO) cells. This resulted in the upregulation of genes that shifted glucose metabolism to aerobic glycolysis [52]. However, although the M2 isoform of pyruvate kinase (M2-PK) required for glycolysis was also upregulated, it remained in the inactive dimeric form due to ROS-mediated oxidation of its cysteine residues. The inactive dimeric form of M2-PK caused the accumulation of phospho-metabolites above phosphoenolpyruvate (PEP) in the glycolytic pathway. These metabolites were channeled to biosynthetic reactions including nucleic acid biosynthesis [53].

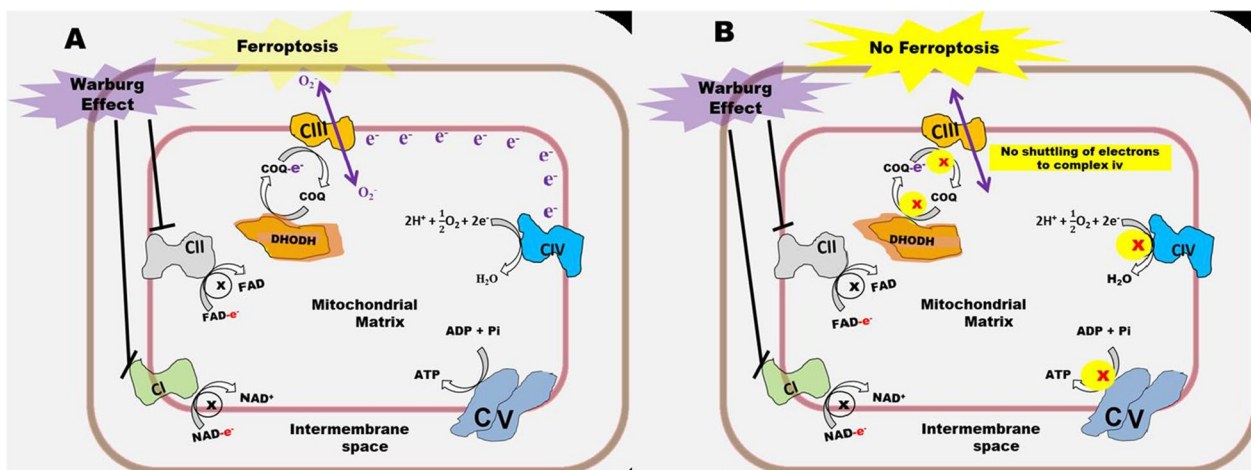
NADH:ubiquinone oxidoreductase subunit A10 (NDUFA10) is a key component of complex I of the ETC. PINK1 loss of function mutation affects complex I activity through the failure of serine 250 of NDUFA10 to become phosphorylated [54]. This is because a phosphorylated NDUFA10 is required for the reduction of ubiquinone by complex I [54]. This suggests that in addition to shifting glucose metabolism towards aerobic glycolysis through an oxidative stress-mediated stabilization of HIF-1, loss of PINK1 activity could affect the ETC by direct inhibition of complex I. The question now arises: between oxidative stress and the inhibition of complex I activity, which of the two plays the most important role in the induction of aerobic glycolysis? A study that

assessed the effect of complex I deficiency and a defect in complex I assembly on the Warburg effect suggest that in the absence of ROS, a deficiency in complex I activity may not be sufficient to induce aerobic glycolysis [31]. Complex I is made up of the matrix arm and the membrane arm. The matrix arm is composed of two modules, the N (NADH binding) and Q (Quinone binding) module while the membrane arm comprises the P (Proton pumping) module. While the P module is encoded by mitochondrial DNA, the N and Q modules are encoded by nuclear DNA. These modules are built independently and later assembled together [55]. This study revealed that a defect in complex I assembly results in excessive ROS generation which resulted in the inhibition of pyruvate dehydrogenase in an AMPK-dependent manner.

Another mechanism through which mitochondrial ROS activates the Warburg effect involves the upregulation of mitochondrial uncoupling proteins (UCPs). Induction of oxidative stress in mouse skin tissues and embryonic fibroblast primary cell culture through SOD2 KO induced the Warburg effect. It was discovered that the oxidative stress-induced the uncoupling of the ETC to ATP synthesis which upregulated glycolysis to compensate for the reduction in ATP generated by the mitochondria [56]. A mechanistic study revealed that SOD2 deficiency caused the nuclear receptor; peroxisome proliferators activated receptors  $\alpha$  (PPAR $\alpha$ ) to upregulate the expression of UCPs. This study also reported an increase in HIF-1 $\alpha$  in response to oxidative stress and it was concluded that antioxidant-deficient mitochondria may serve as early cellular signaling responsible for the activation of the Warburg Effect in cancers.

An observation that contributed to the biochemical description of ferroptosis involves the ability of erastin and RSL3 to selectively inhibit the growth of Ras-mutant cancers by inducing a non-apoptotic iron-dependent regulated cell death. It is therefore conceivable that this mutation drives a metabolic reprogramming that makes tumors selectively susceptible to ferroptosis. RSL3 inhibits GPX4, the key antioxidant enzyme that prevents the onset of ferroptosis while erastin is a voltage-dependent anion channels 2 and 3 (VDAC2/3) and cystine-glutamate antiporter (xCT) inhibitor [2]. Mutation in the RAS family of small GTPases (HRAS, NRAS, and KRAS) affects nearly 30% of tumors [1]. Among these, KRAS-driven cancers are characterized by upregulated glycolysis, glutaminolysis, and nucleotide biosynthesis [57]. Upregulation of nucleotide biosynthesis and glutaminolysis suggests the Warburg effect and an active de novo pyrimidine nucleotide biosynthesis which requires glutamine metabolism to provide aspartate [34, 35]. This assertion is supported by the dependence





**Fig. 4** Warburg effect and its modulation of the anti-ferroptosis function of DHODH. **A** In the absence of functional complexes I and II, DHODH sustains the ETC and  $O_2^-$  generation by shuttling electrons to complex III. Complex III-generated  $O_2^-$  induces oxidative stress that results in ferroptosis. **B** When complexes I and II of the ETC are inhibited by the Warburg effect, DHODH inhibition could cause a complete shutdown of the ETC resulting in the failure of complex III to produce  $O_2^-$ . Differences between A and B has been highlighted in yellow

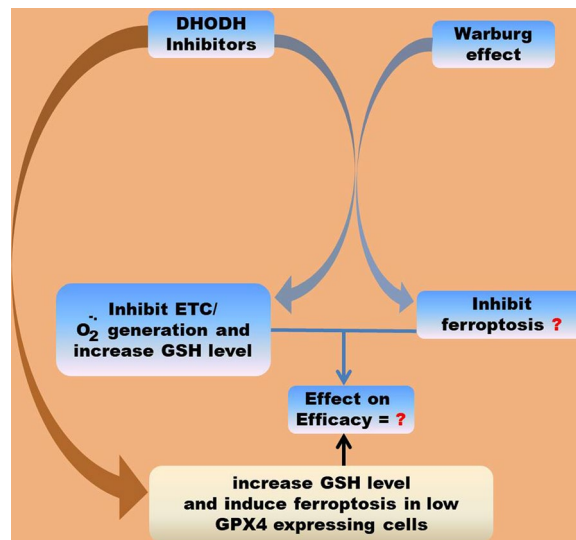
of mutant K-RAS-driven cancers on DHODH [41]. The finding that xCT inhibitors only induce ferroptosis in a fraction of tumor cells sufficiently expressing solute carrier family 7 member 11 (SLC7A11) suggests that in addition to their effect on the functionality of the antioxidant enzyme system, there could be other biochemical mechanisms for ferroptosis induction [58]. It could also imply that to successfully induce ferroptosis, the metabolic phenotype of the tumor must closely resemble that of RAS-mutant cancers where ferroptosis was first induced through SLC7A11 inhibition. Accumulating evidence suggests that one of the metabolic pathways significantly contributing to the sensitivity of RAS-mutant cancers to ferroptosis inducers is de novo pyrimidine nucleotide biosynthesis which under Warburg effect condition, facilitates the shuttling of electrons from complex III to IV.

Oxidation of CoQ10H<sub>2</sub> generated by DHODH to CoQ10 by the ETC could result in complex III-mediated generation of  $O_2^-$  even when the functionality of complex I and II are defective due to the Warburg effect. Generation of  $O_2^-$  by complex III could be required for erastin-induced ferroptosis [13] and possibly, ferroptosis induction in general. Therefore under this condition, if DHODH is inhibited, ferroptosis could equally be inhibited (Fig. 4A and B). The indispensability of a functional DHODH and complex III for tumor growth and the inhibition of complex I and II of the ETC in most tumors by the Warburg effect suggests that under the Warburg effect condition, DHODH-catalyzed reaction could play a significant role in ETC function,  $O_2^-$  generation, and ferroptosis induction. It

is therefore conceivable that in addition to DNA synthesis which consumes cellular GSH, the Warburg effect could affect the anti-ferroptosis role of DHODH.

**Conclusion and future perspective**

DHODH is a versatile drug target involved in the clinical management of many diseases. It is also an attractive drug target in cancer management. As a result of the



**Fig. 5** Schematic representation of conclusion and future perspective. As a result of their potential effect on RNR-catalyzed reaction, a reaction that uses GSH to convert NTPs to dNTPs for DNA synthesis, DHODH inhibitors could increase the intracellular level of GSH. Furthermore, under Warburg effect condition, they could inhibit the ETC and  $O_2^-$  generation. How this affect the onset of ferroptosis and drug efficacy needs to be investigated

importance of de novo pyrimidine nucleotide biosynthesis for tumor growth, an attempt has been made to suppress tumor progression using DHODH inhibitors. One such drug, however, showed unsatisfactory efficacy in phase I and II clinical trials [59, 60]. The role of DHODH in ferroptosis inhibition suggests that its inhibitors could have two complementary mechanisms of action for the suppression of tumor growth that is; inhibition of nucleotide biosynthesis and induction of ferroptosis. However, reports that revealed the indispensability of DHODH-catalyzed reaction for tumor growth, the prevalence of the Warburg effect in solid tumors, and the importance of complex III-generated  $O_2^-$  for ferroptosis induction suggests that under Warburg effect condition, DHODH could be responsible for the generation of  $O_2^-$  by complex III hence making its inhibition to result in suppressing  $O_2^-$  generation and ferroptosis. Inhibiting DHODH could also increase cellular GSH levels thereby making GPX4 to be more efficient in inhibiting ferroptosis. There is therefore a need to investigate the relationship between DHODH inhibitors and how they modulate ferroptosis in solid tumors and how that affects their efficacy. The conclusion and future perspective is schematically represented by Fig. 5.

#### Abbreviations

GPX4	Glutathione peroxidase 4
DHODH	Dihydroorotate dehydrogenase
ETC	Electron transport chain
GSH	Reduced glutathione
RSL3	Ras-selective lethal compound 3
xCT	Cystine-glutamate antiporter
ATP	Adenosine triphosphate
ROS	Reactive oxygen species
S3QEL	Selective inhibitor of superoxide production from the outer Q-binding site of mitochondrial respiratory complex site III
S1QEL	Selective inhibitor of superoxide production from the outer Q-binding site of mitochondrial respiratory complex site I
KO	Knock out
FCCP	Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazine
E1	Alpha 1
E2	Alpha 2
OCR	Oxygen consumption rate
NADH	Reduced nicotinamide adenine dinucleotide
NAD <sup>+</sup>	Oxidized nicotinamide adenine dinucleotide
FADH <sub>2</sub>	Reduced flavin adenine dinucleotide
CoQ10	Ubiquinone
CoQ10H <sub>2</sub>	Ubiquinol
FSP1	Ferroptosis suppressor protein 1
DNA	Deoxyribonucleic acid
NTPs	Nucleosides triphosphates
dNTPs	Deoxynucleosides triphosphates
RNR	Ribonucleotide reductase
GSSG	Oxidized glutathione
AML	Acute myeloid leukemia
AOX	Alternative oxidase
mtDNA	Mitochondrial DNA
GOT1	Glutamate-oxaloacetate transaminase 1
MDH2	Malate dehydrogenase 2
GOT2	Glutamate-oxaloacetate transaminase 2
UMP	Uridine monophosphate

TMP	Thymidine monophosphate
dTMP	Deoxythymidine monophosphate
SLC1A3	Solute carrier family 1 member 3
UVB	Ultraviolet B
SDH	Succinate dehydrogenase
FH	Fumarate hydratase
IDH	Isocitrate dehydrogenase
HIF1- $\alpha$	Hypoxia inducible factor 1 alpha
ODD	Oxygen-dependent degradation domain
PHDs	Prolyl-hydroxylases
NOX	NADPH oxidase
MnSOD	Manganese superoxide dismutase
PINK1	PTEN-induced kinase 1
FOXO3a	Forkhead box O3a
SOD2	Superoxide dismutase 2
M2-PK	M2 isoform of pyruvate kinase
PEP	Phosphoenolpyruvate
NDUFA10	NADH:ubiquinone oxidoreductase subunit A10
AMPK	Adenosine monophosphate kinase
UCPs	Uncoupling proteins
PPAR- $\alpha$	Peroxisome proliferators activated receptors $\alpha$
VDAC2/3	Voltage-dependent anion channels 2 and 3
SLC7A11	Solute carrier family 7 member 11

#### Acknowledgements

Not applicable

#### Author contributions

The Idea was conceived by AA, AA and AX performed the literature search and drafted the manuscript; LW and HX critically revised the article. All authors read and approved the final manuscript.

#### Funding

This work was supported by a grant from the National Natural Science Foundation of China [No. 82172804; 82003232], China International Medical Foundation [No. NTC03932266], Key Program of Jiangsu Provincial Department of Science and Technology [No. BE2019756], Key Program of Jiangsu Commission of Health [No.K2019028], The sixth "333 Talents" Outstanding Young Talents Project of Jiangsu Province and Nasopharyngeal Carcinoma Cohort Program of Nanjing Medical University [No.NMUC2021011A].

#### Availability of data and materials

Not applicable.

#### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

No competing interest.

Received: 9 May 2022 Accepted: 22 December 2022

Published online: 05 May 2023

#### References

- Vigil D, Cherfils J, Rossman KL, Der CJ. Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? *Nat Rev Cancer*. 2010;10(12):842–57.
- Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*. 2012;149(5):1060–72.

3. Mao C, Liu X, Zhang Y, Lei G, Yan Y, Lee H, et al. DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature*. 2021;593(7860):586–90.
4. Yang Wan S, SriRamaratnam R, Welsch Matthew E, Shimada K, Skouta R, Viswanathan Vasanthi S, et al. Regulation of ferroptotic cancer cell death by GPX4. *Cell*. 2014;156(1):317–31.
5. Shibata Y, Yasui H, Higashikawa K, Miyamoto N, Kuge Y. Erastin, a ferroptosis-inducing agent, sensitizes cancer cells to X-ray irradiation via glutathione starvation in vitro and in vivo. *PLoS one*. 2019;14(12):e0225931.
6. Han C, Liu Y, Dai R, Ismail N, Su W, Li B. Ferroptosis and its potential role in human diseases. *Front Pharmacol*. 2020;11:239.
7. Boukalova S, Hubackova S, Milosevic M, Ezrova Z, Neuzil J, Rohlena J. Dihydroorotate dehydrogenase in oxidative phosphorylation and cancer. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866(6): 165759.
8. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009;324(5930):1029–33.
9. Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci*. 2016;41(3):211–8.
10. Nolfi-Donagan D, Braganza A, Shiva S. Mitochondrial electron transport chain: oxidative phosphorylation, oxidant production, and methods of measurement. *Redox Biol*. 2020;37:101674.
11. Greene J, Segaran A, Lord S. Targeting OXPHOS and the electronic transport chain in cancer; molecular and therapeutic implications. *Semin Cancer Biol*. 2022. <https://doi.org/10.1016/j.semcancer.2022.02.002>.
12. Muller FL, Liu Y, Van Remmen H. Complex III releases superoxide to both sides of the inner mitochondrial membrane. *J Biol Chem*. 2004;279(47):49064–73.
13. Homma T, Kobayashi S, Sato H, Fujii J. Superoxide produced by mitochondrial complex III plays a pivotal role in the execution of ferroptosis induced by cysteine starvation. *Arch Biochem Biophys*. 2021;700: 108775.
14. Gao MH, Yi JM, Zhu JJ, Minikes AM, Monian P, Thompson CB, et al. Role of mitochondria in ferroptosis. *Molecular Cell*. 2019;73(2):354.
15. Vučković A-M, Venerando R, Tibaldi E, Bosello Travain V, Roveri A, Bordin L, et al. Aerobic pyruvate metabolism sensitizes cells to ferroptosis primed by GSH depletion. *Free Radical Biol Med*. 2021;167:45–53.
16. Wang L, Duan Q, Wang T, Ahmed M, Zhang N, Li Y, et al. Mitochondrial respiratory chain inhibitors involved in ROS production induced by acute high concentrations of iodide and the effects of SOD as a protective factor. *Oxid Med Cell Longev*. 2015;2015: 217670.
17. Mao C, Liu XG, Zhang YL, Lei G, Yan YL, Lee H, et al. DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature*. 2021;593(7860):586.
18. Doll S, Freitas FP, Shah R, Aldrovandi M, da Silva MC, Ingold I, et al. FSP1 is a glutathione-independent ferroptosis suppressor. *Nature*. 2019;575(7784):693.
19. Hollenstein M, Smith CC, Rätz M. Nucleoside triphosphates—from synthesis to biochemical characterization. *J Vis Exp*. 2014;86:51385.
20. Desler C, Durhuus JA, Hansen TL-L, Anugula S, Zelander NT, Bøggild S, et al. Partial inhibition of mitochondrial-linked pyrimidine synthesis increases tumorigenic potential and lysosome accumulation. *Mitochondrion*. 2022;64:73–81.
21. Tarangelo A, Rodencal J, Kim JT, Magtanong L, Long JZ, Dixon SJ. Nucleotide biosynthesis links glutathione metabolism to ferroptosis sensitivity. *Life Sci Alliance*. 2022. <https://doi.org/10.26508/lsa.2021011157>.
22. Robinson AD, Eich ML, Varambally S. Dysregulation of de novo nucleotide biosynthetic pathway enzymes in cancer and targeting opportunities. *Cancer Lett*. 2020;470:134–40.
23. Khutornenko AA, Roudko VV, Chernyak BV, Vartapetian AB, Chumakov PM, Evstafieva AG. Pyrimidine biosynthesis links mitochondrial respiration to the p53 pathway. *Proc Natl Acad Sci USA*. 2010;107(29):12828–33.
24. Zhang YP, Luo T, Ding XY, Chang YT, Liu CX, Zhang YQ, et al. Inhibition of mitochondrial complex III induces differentiation in acute myeloid leukemia. *Biochem Biophys Res Commun*. 2021;547:162–8.
25. Martinez-Reyes I, Cardona LR, Kong H, Vasan K, McElroy GS, Werner M, et al. Mitochondrial ubiquinol oxidation is necessary for tumour growth. *Nature*. 2020;585(7824):288.
26. Bajzikova M, Kovarova J, Coelho AR, Boukalova S, Oh S, Rohlenova K, et al. Reactivation of dihydroorotate dehydrogenase-driven pyrimidine biosynthesis restores tumor growth of respiration-deficient cancer cells. *Cell Metab*. 2019;29(2):399–416.e10.
27. Dong LF, Kovarova J, Bajzikova M, Bezawork-Geleta A, Svec D, Endaya B, et al. Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondria! DNA-deficient cancer cells. *Elife*. 2017;6:e22187.
28. Tan AS, Baty JW, Dong LF, Bezawork-Geleta A, Endaya B, Goodwin J, et al. Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. *Cell Metab*. 2015;21(1):81–94.
29. Qu CB, Zhang SS, Wang WS, Li M, Wang YJ, van der Heijde-Mulder M, et al. Mitochondrial electron transport chain complex III sustains hepatitis E virus replication and represents an antiviral target. *FASEB J*. 2019;33(1):1008–19.
30. Laskey RA, Fairman MP, Blow JJ. S phase of the cell cycle. *Science*. 1989;246(4930):609–14.
31. Desquiret-Dumas V, Leman G, Wetterwald C, Chupin S, Lebert A, Khiati S, et al. Warburg-like effect is a hallmark of complex I assembly defects. *BBA-Mol Basis Dis*. 2019;1865(9):2475–89.
32. Holley AK, Dhar SK, St Clair DK. Curbing cancer's sweet tooth: is there a role for MnSOD in regulation of the Warburg effect? *Mitochondrion*. 2013;13(3):170–88.
33. Birsoy K, Wang T, Chen WW, Freinkman E, Abu-Remaileh M, Sabatini DM. An essential role of the mitochondrial electron transport chain in cell proliferation is to enable aspartate synthesis. *Cell*. 2015;162(3):540–51.
34. Yoo HC, Yu YC, Sung Y, Han JM. Glutamine reliance in cell metabolism. *Exp Mol Med*. 2020;52(9):1496–516.
35. Yang LF, Venneti S, Nagrath D. Glutaminolysis: a Hallmark of Cancer Metabolism. In: Yarmush ML, editor. Annual review of biomedical engineering, vol 19, 192017: 163–194.
36. Khutornenko AA, Dalina AA, Chernyak BV, Chumakov PM, Evstafieva AG. The role of dihydroorotate dehydrogenase in apoptosis induction in response to inhibition of the mitochondrial respiratory chain complex III. *Acta Naturae*. 2014;6(1):69–75.
37. Desbats MA, Lunardi G, Doimo M, Trevisson E, Salviati L. Genetic bases and clinical manifestations of coenzyme Q10 (CoQ10) deficiency. *J Inherit Metab Dis*. 2015;38(1):145–56.
38. Brodsky AN, Odenwelder DC, Harcum SW. High extracellular lactate causes reductive carboxylation in breast tissue cell lines grown under normoxic conditions. *Plos One*. 2019;14(6):e0213419.
39. Garcia-Bermudez J, Baudrier L, La K, Zhu XG, Fidelin J, Sviderskiy VO, et al. Aspartate is a limiting metabolite for cancer cell proliferation under hypoxia and in tumours. *Nat Cell Biol*. 2018;20(10):1228.
40. Stuchebrukhov AA. Redox-driven proton pumps of the respiratory chain. *Biophys J*. 2018;115(5):830–40.
41. Koundinya M, Sudhalter J, Courjaud A, Lionne B, Touyer G, Bonnet L, et al. Dependence on the pyrimidine biosynthetic enzyme DHODH is a synthetic lethal vulnerability in mutant KRAS-driven cancers. *Cell Chem Biol*. 2018;25(6):705.
42. Hosseini M, Dousset L, Mahfouf W, Serrano-Sanchez M, Redonnet-Vernhet I, Mesli S, et al. Energy metabolism rewiring precedes UVB-induced primary skin tumor formation. *Cell Rep*. 2018;23(12):3621–34.
43. Cardaci S, Ciriolo MR. TCA cycle defects and cancer: when metabolism tunes redox state. *Int J Cell Biol*. 2012;2012: 161837.
44. Lorendeau D, Rinaldi G, Boon R, Spincemaille P, Metzger K, Jager C, et al. Dual loss of succinate dehydrogenase (SDH) and complex I activity is necessary to recapitulate the metabolic phenotype of SDH mutant tumors. *Metab Eng*. 2017;43:187–97.
45. Tyrakis PA, Yurkovich ME, Sciacovelli M, Papachristou EK, Bridges HR, Gaude E, et al. Fumarate hydratase loss causes combined respiratory chain defects. *Cell Rep*. 2017;21(4):1036–47.
46. McCarty MF, Barroso-Aranda J, Contreras F. Oxidative stress therapy for solid tumors—a proposal. *Med Hypotheses*. 2010;74(6):1052–4.
47. Jiang Y, Wu GH, He GD, Zhuang QL, Xi QL, Zhang B, et al. The effect of silencing HIF-1 $\alpha$  gene in BxPC-3 cell line on glycolysis-related gene expression, cell growth, invasion, and apoptosis. *Nutr Cancer*. 2015;67(8):1314–23.
48. He WG, Batty-Stuart S, Lee JE, Ohh M. HIF-1  $\alpha$  hydroxyprolines modulate oxygen-dependent protein stability via single VHL interface with comparable effect on ubiquitination rate. *J Mol Biol*. 2021;433(22):17.

49. Patten DA, Lafleur VN, Robitaille GA, Chan DA, Giaccia AJ, Richard DE. Hypoxia-inducible factor-1 activation in nonhypoxic conditions: the essential role of mitochondrial-derived reactive oxygen species. *Mol Biol Cell*. 2010;21(18):3247–57.
50. Shi DY, Xie FZ, Zhai C, Stern JS, Liu Y, Liu SL. The role of cellular oxidative stress in regulating glycolysis energy metabolism in hepatoma cells. *Mol Cancer*. 2009;8(1):1–5.
51. Sanjuan-Pla A, Cervera AM, Apostolova N, Garcia-Bou R, Victor VM, Murphy MP, et al. A targeted antioxidant reveals the importance of mitochondrial reactive oxygen species in the hypoxic signaling of HIF-1 alpha. *FEBS Lett*. 2005;579(12):2669–74.
52. Agnihotri S, Golbourn B, Huang X, Remke M, Younger S, Cairns RA, et al. PINK1 is a negative regulator of growth and the Warburg effect in glioblastoma. *Can Res*. 2016;76(16):4708–19.
53. Zahra K, Dey T, Ashish Mishra SP, Pandey U. Pyruvate kinase M2 and cancer: the role of PKM2 in promoting tumorigenesis. *Front Oncol*. 2020;10:159.
54. Morais VA, Haddad D, Craessaerts K, De Bock PJ, Swerts J, Vilain S, et al. PINK1 loss-of-function mutations affect mitochondrial complex I activity via NdufA10 ubiquinone uncoupling. *Science*. 2014;344(6180):203–7.
55. Sánchez-Caballero L, Guerrero-Castillo S, Nijtmans L. Unraveling the complexity of mitochondrial complex I assembly: a dynamic process. *Biochimica et Biophysica Acta (BBA) Bioenergetics*. 2016;1857(7):980–90.
56. Xu Y, Miriyala S, Fang F, Bakthavatchalu V, Noel T, Schell DM, et al. Manganese superoxide dismutase deficiency triggers mitochondrial uncoupling and the Warburg effect. *Oncogene*. 2015;34(32):4229–37.
57. Kawada K, Toda K, Sakai Y. Targeting metabolic reprogramming in KRAS-driven cancers. *Int J Clin Oncol*. 2017;22(4):651–9.
58. Zheng J, Sato M, Mishima E, Sato H, Proneth B, Conrad M. Sorafenib fails to trigger ferroptosis across a wide range of cancer cell lines. *Cell Death Dis*. 2021;12(7):698.
59. Arteaga CL, Brown TD, Kuhn JG, Shen HS, O'Rourke TJ, Beougher K, et al. Phase I clinical and pharmacokinetic trial of Brequinar sodium (DuP 785; NSC 368390). *Cancer Res*. 1989;49(16):4648–53.
60. Peters GJ. Re-evaluation of Brequinar sodium, a dihydroorotate dehydrogenase inhibitor. *Nucleosides Nucleotides Nucleic Acids*. 2018;37(12):666–78.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

