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DHODH-mediated mitochondrial redox homeostasis: a novel ferroptosis regulator and promising therapeutic target

Jinghao Cao [a,1], Xi Chen [a,1], Lulu Chen [a,1], Yajuan Lu [a], Yunyi Wu [a], Aoli Deng [a], Feifan Pan [a], Hangqi Huang [a], Yingchao Liu [a], Yanchun Li [b,*], Xiangmin Tong [b,**], Jing Du [a,***]

[a] Laboratory Medicine Center, Department of Clinical Laboratory, Zhejiang Provincial People's Hospital (Affiliated People's Hospital), Hangzhou Medical College, Hangzhou, Zhejiang, 310014, China

[b] Department of Clinical Laboratory, Affiliated Hangzhou First People's Hospital, School of Medicine, Westlake University, Hangzhou, Zhejiang, 310006, China

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ABSTRACT

Ferroptosis is a distinct form of regulated cell death characterized by iron-dependent lipid peroxidation, which plays a critical role in the pathogenesis of various diseases, including ischemic tissue injury, infectious diseases, neurodegenerative disorders, and cancer. The regulatory mechanisms underlying ferroptosis involve a complex interplay of multiple subcellular organelles, orchestrating iron homeostasis, lipid metabolism, and the generation of reactive oxygen species (ROS) that drive peroxidation processes, ultimately leading to membrane damage and cell death. Numerous antioxidant systems play pivotal roles in regulating and preventing ferroptosis, among which the recently identified mitochondrial inner membrane enzyme dihydroorotate dehydrogenase (DHODH) represents a novel therapeutic target for ferroptosis intervention. This systematic review comprehensively elucidates several key cellular defense mechanisms against ferroptosis that counteract ROS-driven peroxidation and operate through distinct subcellular localizations. We particularly focus on delineating the molecular mechanisms by which DHODH regulates ferroptosis, with special emphasis on its role in suppressing mitochondrial lipid peroxidation. Furthermore, we systematically evaluate the therapeutic potential of DHODH inhibitors in oncology, virology, and immune-inflammatory disorders. By integrating ferroptosis biology with DHODH-mediated cytoprotective networks, this review aims to provide mechanistic insights and novel therapeutic strategies for cancer and oxidative stress-related disorders.

1. Introduction

In 2012, Dixon and colleagues first conceptualized ferroptosis, a novel form of regulated cell death triggered by the compound erastin. This iron-dependent form of regulated cell death, termed ferroptosis, involves the pathological accumulation of lipid hydroperoxides, primarily orchestrated through interconnected dysregulation of iron homeostasis, lipid biosynthesis, and oxidative stress pathways across multiple cellular compartments [1,2]. The resulting oxidative membrane damage ultimately leads to irreversible cell death. Distinct from necrosis (cytoplasmic swelling), apoptosis (nuclear condensation), and autophagy (double-membrane vesicles), ferroptosis exhibits unique mitochondrial pathology, including organelle contraction, heightened membrane compaction, and disruption of cristae integrity [3]. Ferroptosis is fueled by iron-dependent lipid peroxidation, with its underlying mechanism regulated through the dynamic interaction of iron homeostasis, reactive oxygen species (ROS) production, and perturbation of antioxidant defense systems, particularly the glutathione peroxidase 4 (GPX4) pathway and the ferroptosis suppressor protein 1 (FSP1)-coenzyme Q_{10} (CoQ_{10}) axis [4]. Ferroptosis has been identified as a pivotal pathogenic driver across diverse pathologies, notably in oncology, neurodegenerative diseases, and cardiovascular disorders, where targeting this process holds significant therapeutic potential [5]. Emerging therapeutic strategies targeting ferroptosis have reached advanced preclinical stages, exemplified by small-molecule inhibitors (Ferrostatin-1 derivatives and GPX4-targeting agents), while

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: lycmed@163.com (Y. Li), tongxiangmin@163.com (X. Tong), dujing1@hmc.edu.cn (J. Du).
[1] These authors contributed equally: Jinghao Cao, Xi Chen, Lulu Chen.

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nanoparticle-mediated delivery platforms demonstrate enhanced therapeutic precision through iron homeostasis regulatory mechanisms [6]. Ferroptosis mechanism has gained prominence as a key therapeutic target, stimulating multidisciplinary research to uncover novel therapeutic avenues through its distinct regulatory pathways in lipid peroxidation and iron homeostasis [7].

Human dihydroorotate dehydrogenase (DHODH) functions as an integral inner mitochondrial membrane flavoprotein, mediating the fourth enzymatic step in pyrimidine de novo synthesis through its critical redox activity [8]. DHODH has emerged as a key therapeutic target for hyperproliferative disorders, parasitic infections (including malaria), and viral diseases [9], and was initially developed for the treatment of autoimmune disorders, including rheumatoid arthritis and multiple sclerosis. DHODH inhibitors have demonstrated significant therapeutic potential in oncology, emerging as a promising class of anticancer agents [10]. Emerging evidence highlights that DHODH suppresses mitochondrial ferroptosis by restoring coenzyme Q_{10} ($CoQH_2$) levels through mitochondrial membrane electron transfer, establishing this redox pathway as a key element in cellular defense against ferroptosis [8].

This comprehensive review systematically elucidates the molecular mechanisms underlying ferroptosis, integrating its initiation, progression, regulatory networks, and cellular defense mechanisms. Particular emphasis is placed on DHODH, a mitochondrial enzyme that mitigates ferroptosis by reducing CoQ_{10} to $CoQH_2$—a lipid-soluble antioxidant that neutralizes peroxidation intermediates and protects mitochondrial membranes from oxidative damage. This DHODH-mediated defense mechanism operates independently of cytosolic GPX4 and plasma membrane-bound FSP1, highlighting its unique role in stabilizing mitochondrial membrane integrity and redox homeostasis. Furthermore, we critically evaluate the therapeutic potential of modulating ferroptosis through DHODH inhibition or activation, highlighting its clinical applications in combined cancer therapies, antiviral treatments, autoimmune disease management, and inflammatory disease intervention. By synthesizing recent advances in ferroptosis research and DHODH-mediated cytoprotection, this review aims to provide mechanistic insights and novel therapeutic strategies for cancer and oxidative stress-related disorders.

2. Definition and mechanism of ferroptosis

Ferroptosis primarily revolves around iron-mediated lipid hydroperoxide (LOOH) accumulation, initiating uncontrolled lipid peroxidation cascades and compromising LOOH degradation pathways. This process destabilizes intracellular redox homeostasis, induces oxidative damage to biological macromolecules, and ultimately triggers regulated cell death. Notably, LOOHs serve as pivotal mediators in ferroptotic signaling, and pharmacological inhibition of LOOH formation represents a promising therapeutic strategy for preventing ferroptosis [11]. Nevertheless, the exact molecular mechanisms governing the terminal phase of ferroptosis remain unclear, necessitating further studies to

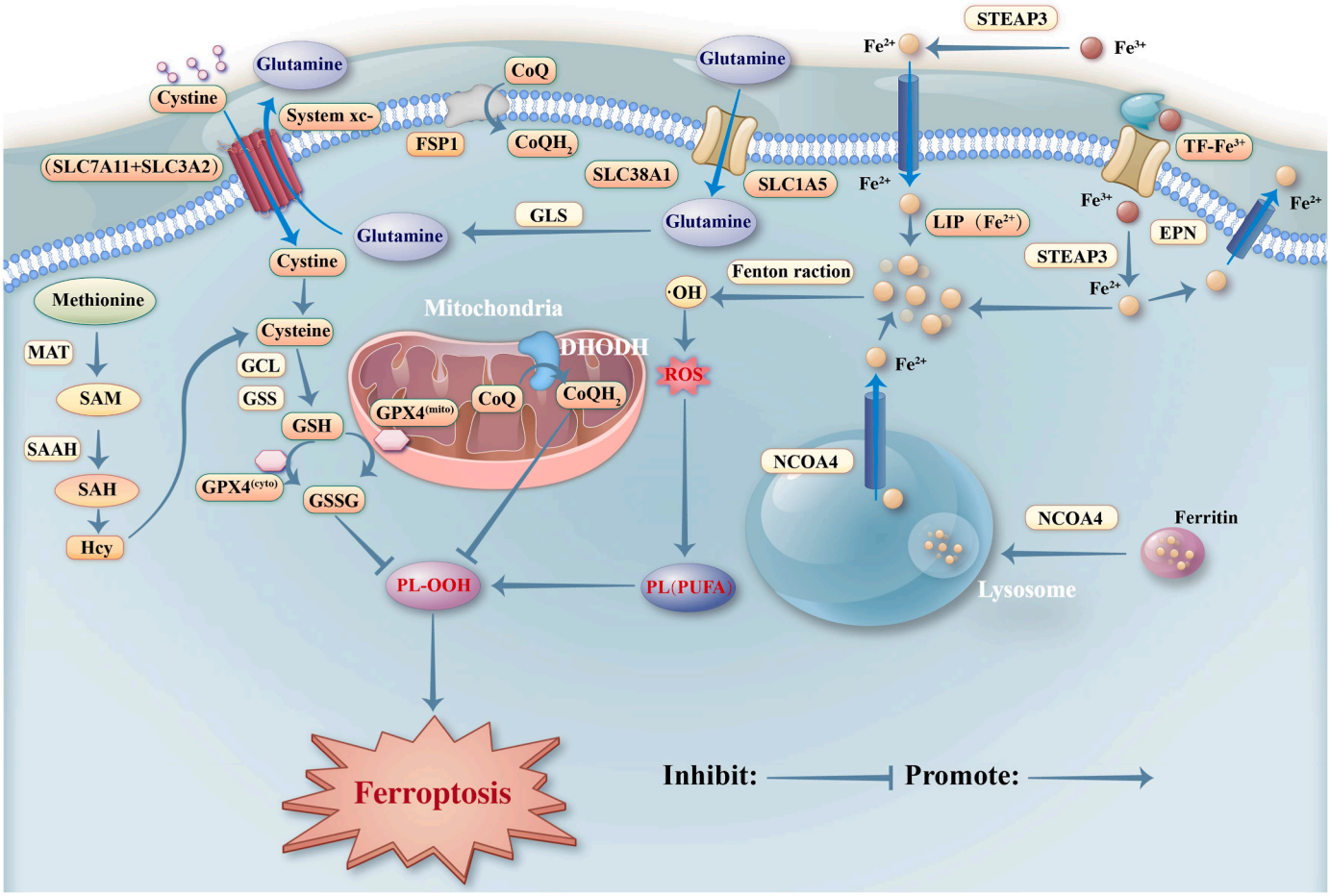

Fig. 1. The molecular mechanisms underlying ferroptosis and their interplay with cellular antioxidant defense systems
Ferroptosis is fundamentally characterized by iron-dependent lipid peroxidation, which constitutes the core biochemical feature of this regulated cell death modality. The principal mechanisms driving ferroptosis involve the accumulation of redox-active iron and subsequent peroxidation of PUFAs in cellular membranes. This peroxidation cascade is initiated and propagated through various oxidative stress stimuli that overwhelm cellular antioxidant defenses. Multiple evolutionarily conserved antioxidant systems have been identified as critical regulators of ferroptosis, including the System Xc-GSH-GPX4 pathway, transsulfuration pathway, mevalonate pathway, FSP1-CoQ_{10} pathway and DHODH-$CoQH_2$ pathway.

define the concluding steps in this regulated cell death process.

Intuitively, ferroptosis manifests as GSH pool exhaustion and subsequent GPX4 inactivation. This critical impairment of the GPX4-catalyzed reduction pathway results in the accumulation of lipid peroxides due to their impaired metabolic clearance [3]. The accumulation of intracellular free Fe^{2+} initiates a cascade of oxidative events through Fenton chemistry, catalyzing lipid oxidation and generating substantial ROS (Fig. 1). This iron-dependent oxidative stress amplifies lipid peroxidation sensitivity, thereby potentiating ferroptosis through a self-amplifying cycle of oxidative damage [3,11].

The process of ferroptosis is driven by the interplay of the following key aspects: (1) Iron homeostasis dysregulation. Iron loading: Iron is a critical mediator of lipid peroxidation, and excess Fe^{2+} directly or indirectly promotes lipid peroxidation, triggering ferroptosis [12]. Iron uptake: Cells rely on transferrin and transferrin receptor for Fe^{3+} uptake, as well as Fe^{2+} through divalent metal transporter 1 (DMT1). Iron uptake affects cell sensitivity to ferroptosis [13]. Iron storage: Iron is stored as ferritin, and its release as labile iron drives ferroptosis [14]. Iron regulation: Iron regulatory proteins (IRPs) modulate iron homeostasis through IRE-binding-mediated translational regulation of key iron metabolism genes [15]. (2) Lipid peroxidation cascade. ROS-driven initiation: The iron-catalyzed peroxidation of phospholipids containing arachidonic or adrenic acid, propelled by GPX4 dysfunction and Fenton reactions, emerges as the quintessential biochemical signature of ferroptosis. In this detrimental process, ROS functions as both the initiators that spark the reaction and the amplifiers that exacerbate its progression. The magnitude of LOOH accumulation quantitatively reflects oxidative stress intensity [16,17]. Secondary toxicity: LOOH degradation generates reactive aldehydes (4-hydroxynonenal, malondialdehyde) that induce protein crosslinking and functional impairment, exacerbating cell death [11]. (3) Antioxidant defense collapse. GSH system dysregulation: The GSH system is essential for cellular antioxidant defense. Reducing GSH levels impairs the ability to detoxify lipid peroxides, thereby promoting ferroptosis [18]. GPX4, an important enzyme, employs GSH to scavenge lipid peroxides, and its inhibition exacerbates ferroptosis [19]. FSP1-CoQ_{10} axis: FSP1 sustains $CoQH_2$ regeneration via NADPH, providing parallel protection against membrane lipid peroxidation [20]. NRF2-KEAP1 axis: The Nuclear factor erythroid 2-related factor 2 (NRF2)-Kelch-like ECH-associated protein 1 (KEAP1) axis coordinates adaptive responses to iron and oxidative stress, modulating ferroptosis thresholds [21]. (4) Membrane and mitochondrial failure. Cell membrane damage: With lipid peroxide buildup, cell membrane integrity is disrupted. The oxidation of membrane phospholipids leads to membrane rupture, loss of cellular functions, and eventual cell death [22]. Impaired mitochondrial function: Mitochondria are central mediators of ferroptosis through ROS overproduction. Mitochondrial dysfunction, including damage to the mitochondrial membrane and impaired energy metabolism, exacerbates the oxidative damage, leading to ferroptosis [23]. Mitochondria are also involved in iron storage and superoxide production, further contributing to this process [24]. Mitochondrial protein CDGSH Iron Sulfur Domain-containing protein 3 (CISD3) deficiency drives metabolic reprogramming toward glutaminolysis, fueling mitochondrial electron transport chain (ETC) substrate supply and culminating in oxidative stress and ferroptosis [25].

Substances inducing ferroptosis fall into the following categories by their mechanisms of action and pathways: (1) Core pathway inhibitors. Substances that inhibit GPX4: for example, RSL3 directly inhibits the activity of GPX4 and is a commonly used ferroptosis inducer in research. FIN56 exerts two mechanisms of action in inducing ferroptosis. First, it promotes the degradation of GPX4 directly; second, it combines with squalene synthase, causing endogenous antioxidant CoQ_{10}. Cystamine, a disulfide-containing small-molecule compound, promotes ferroptosis via a dual mechanism involving GSH depletion and direct inhibition of GPX4 activity [26]. System Xc^- blockers: Erastin induces lipid peroxidation and triggers ferroptosis by inhibiting system Xc^-, reducing intracellular GSH levels [27]. Like Erastin, Imidazole Ketone Erasti (IKE) inhibits system Xc^-, depletes GSH, and triggers ferroptosis via lipid peroxidation [28]. (2) Substances that increase iron loading: Dihydroartemisinin induces ferroptosis through autophagy-dependent ferritin degradation [22,29]. Ehrlich ascitic carcinoma is an anticancer drug that elevating intracellular iron levels and suppressing antioxidant defenses [30]. Substances that modification of iron homeostasis, for instance, knocking out ferritin heavy chain 1 (FTH1) or overexpressing transferrin receptor 1 (TFR1), can significantly shift intracellular iron pools, which in turn modulate susceptibility to ferroptosis [31]. (3) Substances that promote oxidative stress. For example, at high doses, Aspirin may induce ferroptosis by promoting lipid peroxidation and the generation of ROS [32]. (4) Substances that induce mitochondrial dysfunction: Sorafenib exerts its *anti*-HCC efficacy partially through mitochondrial function impairment [24]. Carbonyl cyanide-p-(trifluoromethoxy)phenylhydrazone, triggers mitochondrial dysfunction, enhances ROS production, and facilitates iron release, ultimately promoting the onset of ferroptosis [33]. (5) Several traditional Chinese medicines or their components that harbor the potential to promote ferroptosis. For example, Artemisinin derivatives suppress proliferation via autophagy activation [34] and simultaneously drive ferroptosis through PRIM2/SLC7A11 axis disruption [35]. Ginsenoside, a Ginseng genus-specific steroidal compound, displays diverse bioactivities, such as immunoregulatory [36], anti-inflammatory [37,38], and antioxidative effects [39], along with antitumor properties [40]. Specifically, Rh4 suppresses colorectal cancer (CRC) cell proliferation through ROS/p53 pathway activation, which elevates p53 to induce autophagy and concomitantly inhibit GPX4/SLC7A11, thereby promoting ferroptosis [41]. (6) Nanomaterials: For example, Iron-based nanoparticles induce ferroptosis by catalyzing Fenton reaction-driven hydroxyl radical generation [42–44]. The multifunctional nanoparticle drug GDYO@SP94/DOX-Fe2+/sorafenib/SLC7A11-i (MNMG) enhances targeting specificity, improves structural stability, and continuously induces ferroptosis in tumor cells [45].

In conclusion, ferroptosis is a regulated cell death mediated by interconnected molecular networks regulating iron metabolism and redox balance. These regulatory interactions critically determine cellular vulnerability to ferroptosis by maintaining iron homeostasis and oxidative stress balance. Deciphering its mechanistic underpinnings expands our comprehension of cell death regulation and unveils therapeutic opportunities for cancer and oxidative stress-related disorders.

3. Defense system of ferroptosis

As previously described, ferroptosis represents a distinct form of regulated cell death mediated by iron-dependent accumulation of lipid peroxides. Multiple interconnected antioxidant defense systems critically regulate ferroptosis. These include the System Xc^-–GSH–GPX4 axis, transsulfuration pathway, mevalonate pathway, FSP1–CoQ_{10} system, DHODH–$CoQH_2$ pathway, and GTP cyclohydrolase-1 (GCH1)–tetrahydrobiopterin (BH4) pathway. Multiple interconnected antioxidant defense systems have been identified as critical regulators of ferroptosis, including the System Xc-GSH-GPX4 axis, transsulfuration pathway, mevalonate pathway, FSP1-CoQ_{10} system, DHODH-$CoQH_2$ pathway, and GTP cyclohydrolase-1 (GCH1)-tetrahydrobiopterin (BH4) pathway. These molecular networks collectively maintain redox homeostasis by regulating lipid peroxidation and preventing ferroptosis [46]. The System Xc-GSH-GPX4 axis serves as the primary defense mechanism against ferroptosis, with GPX4 functioning as a crucial GSH-dependent phospholipid hydroperoxidase in both cytosolic and mitochondrial compartments [47]. Parallel to this system, the FSP1-CoQ_{10} pathway provides an independent antioxidant mechanism through the action of plasma membrane-localized FSP1, a CoQ_{10} oxidoreductase that operates independently of GSH [48]. More recently, identifying the mitochondrial DHODH-$CoQH_2$ pathway has revealed a novel connection between ferroptosis regulation and nucleotide metabolism. This pathway exerts

its protective effects through DHODH-mediated generation of reduced $CoQH_2$ in the inner mitochondrial membrane, offering new therapeutic opportunities for ferroptosis modulation [49].

3.1. Molecular mechanisms of GPX4 in ferroptosis and therapeutic potential in disease

In cells, GPX4 is the most prominent one in detoxifying lipid peroxides in the cell cytoplasm, playing a crucial role in inhibiting lipid peroxidation and preventing ferroptosis [50,51]. The GPX4-GSH redox axis defends against ferroptosis by enzymatically eliminating phospholipid hydroperoxides (PLOOH) through two-electron reduction to phospholipid alcohols (PLOH) [50]. To date, GPX4 is currently recognized as the sole enzyme capable of directly reducing complex lipid hydroperoxides (e.g., phosphatidyl hydroperoxides) within cellular membranes. The regulation of GPX4 activity is fundamentally governed by the GSH antioxidant system. In this pathway, GSH donates essential electrons to GPX4, reducing phospholipid hydroperoxides to nontoxic phospholipid alcohols and producing oxidized glutathione (GSSG). Glutathione reductase regenerates GSH from GSSG, requiring NADPH as the electron donor. This dependence makes NADPH availability a potential predictor of ferroptosis susceptibility. The de novo synthesis of GSH itself is mediated by cytosolic enzymes utilizing three amino acid precursors: glutamate, cysteine, and glycine. Cysteine availability is particularly important, which is determined by System Xc^-, the cystine/glutamate antiporter that facilitates cellular uptake of cystine for subsequent reduction to cysteine. This makes System Xc^- a critical ferroptosis checkpoint, as its pharmacological inhibition potently induces ferroptosis. Emerging therapeutic strategies have leveraged these mechanistic insights [52].

GPX4 plays a key role in a variety of physiological functions. Genetic ablation of GPX4 results in complete embryonic lethality in mice, underscoring its indispensable role in maintaining redox homeostasis during embryogenesis. Knockout of GPX4 by conditional induction in adult mice impairs the integrity of multiple organs, such as the brain and kidney. Deficiency of GPX4 in specific cell types leads to cell death and dysfunction in the retina, brain, liver, and reproductive tissues [53], whereas upregulation of GPX4 expression could attenuate cerebral hemorrhage-related neuronal damage via inhibiting ferroptosis [54]. In addition, GPX4 is critical in hematopoietic cells, such as T cells, erythroid precursors, and myeloid cells [53].

Previous research has demonstrated that nobiletin, a polymethoxyflavone of plant origin, alleviates ferroptosis in septic liver injury via regulating gut microbiota and boosting NRF2-GPX4 axis, underscoring its promise as a protective agent against sepsis [55]. Astragaloside IV, a key bioactive constituent of Astragalus membranaceus, alleviates PM2.5-induced lung injury via the NRF2/SLC7A11/GPX4 axis by modulating ferroptosis signaling [56]. Targeting the stability or post-translational modification mechanisms of GPX4 holds promise as an innovative strategy to enhance ferroptosis sensitivity and overcome tumor drug resistance. Future combination therapies integrating immunotherapy or metabolic reprogramming are anticipated to unlock their clinical potential further.

3.2. Molecular mechanisms of FSP1 in ferroptosis and therapeutic potential in disease

FSP1, also referred to as apoptosis-inducing factor mitochondria-associated protein 2, has emerged as a critical inhibitor of ferroptosis. Although it shares a resemblance in amino acid sequence with the human apoptosis-inducing factor, its primary activity occurs in the plasma membrane. Notably, FSP1 safeguards against ferroptosis stemming from GPX4 deletion. Functioning as a flavoprotein redox enzyme, FSP1 likely modulates oxidation-reduction activities. Recent CRISPR/Cas9-based screenings have identified FSP1 as a novel player in ferroptosis resistance [57]. This discovery has shed light on FSP1's role in inhibiting ferroptosis through a distinct mechanism involving CoQ_{10} mediation.

Unlike conventional GSH-dependent antioxidant systems, FSP1 mediates ferroptosis suppression through NAD(P)H-dependent catalysis of CoQ_{10} reduction, converting CoQ_{10} to its reduced form, $CoQ_{10}H_2$, thereby neutralizing lipid peroxidation and preserving cellular membrane integrity [58,59]. CoQ_{10} stands as a pivotal constituent of the FSP1 pathway, distributed across the membranes of mammalian cells. Within the plasma membrane, it functions as a redox carrier, contributing to the elimination of lipid superoxide free radicals and acting as an endogenous fat-soluble antioxidant. Studies underscore CoQ_{10}'s potential to suppress ferroptosis, and its therapeutic benefits extend to various human ailments, including cardiomyopathy, Parkinson's disease, and diabetes [52].

Studies have demonstrated that pharmacological targeting of FSP1 strongly synergizes with GPX4 inhibitors to induce ferroptosis in diverse cancers, including NSCLC [60] and breast cancer [58]. Therefore, these two pathways play complementary and synergistic roles in maintaining cellular redox balance [58]. The iFSP1, first FSP1-specific inhibitor, competitively inhibits FSP1 enzymatic activity by blocking the reduction of CoQ_{10} to $CoQH_2$, but cannot directly induce ferroptosis [60]. Due to limitations in iFSP1's in vivo application and its off-target effects at high concentrations [61], it was not further developed as a clinical drug. Notably, iFSP1 exhibits synergistic effects with GPX4 inhibitors in enhancing ferroptosis [60]. The modified analog icFSP1 non-competitively inhibits FSP1 by triggering its plasma membrane dissociation and phase separation into condensates, disrupting antioxidant function to induce ferroptosis. Future efforts should focus on developing next-generation FSP1 inhibitors based on the phase separation mechanism and exploring their synergistic strategies with GPX4 inhibitors or immunotherapy, which holds promise to overcome drug resistance and expand clinical applications.

3.3. DHODH inhibits mitochondrial lipid peroxides and therapeutic implications

DHODH is a dehydrogenase localized to the inner mitochondrial membrane. DHODH and mitochondrial GPX4 synergistically prevent mitochondrial lipid peroxide accumulation by reducing CoQ to $CoQH_2$. Thus, DHODH/GPX4 inhibition promotes mitochondrial lipid peroxidation and ferroptosis, supporting their targeting in cancer therapy [48]. Previous studies have demonstrated that, compared to non-targeted antioxidants, mitochondria-targeted antioxidants exhibit reduced rescue efficiency in GPX4-inactivated cells. This finding suggests that mitchondria-external compartments (e.g., endoplasmic reticulum or plasma membrane) can also produce substantial amounts of lipid peroxides, which trigger lethal cell death signals [62]. DHODH can inhibit ferroptosis through several pathways, as detailed later.

3.4. P53 transcriptionally repressing SLC7A11 and sensitizing cells to ferroptosis

The Xc^- system is composed of two subunits: SLC7A11, which serves as the functional light chain, and SLC3A2, the heavy chain. Together, they constitute the Xc^- system. p53 is a tumor suppressor, but recent studies have shown it regulates various other cellular processes, such as antioxidant defense and metabolism. Prior studies show that p53 transcriptionally represses SLC7A11 expression and triggers ferroptosis [63]. Acetylation of p53 at lysine 101 (human) or lysine 98 (mouse) activates its function and enhances ferroptosis induction [64]. While p53 deacetylation minimally affects expression, it significantly modulates transcriptional control of targets such as SLC7A11 [65]. In addition, SIRT1 deacetylates p53, abrogating its repression of SLC7A11 transcription to increase SLC7A11 expression and inhibit ferroptosis [66].

A prominent class of SLC7A11-targeting therapeutic agents functions

by directly inhibiting its amino acid transport activity, thereby depleting intracellular GSH levels. This category encompasses compounds such as sulfasalazine, erastin derivatives (including imidazole ketone erastin), and HG106 [67]. For example, HG106 selectively induces ferroptosis in KRAS-mutant lung adenocarcinoma cells by depleting intracellular cysteine via SLC7A11 inhibition, thereby amplifying oxidative stress through GSH depletion and activating endoplasmic reticulum (ER) stress via PERK/eIF2α/CHOP signaling. Preclinical studies in KRAS-mutant lung adenocarcinoma xenografts and genetically engineered mouse models demonstrate that HG106 reduces tumor volume compared to controls, validating its therapeutic efficacy [68]. Previous studies have demonstrated that p53 directly inhibits the transcription of *SLC7A1*1 by binding to DNA response elements within its promoter region. Downregulation of SLC7A11 results in GSH depletion, reduced activity of GPX4, uncontrolled lipid peroxidation, and ultimately induces ferroptosis [69]. For example, nobiletin activates p53, which inhibits the expression of SLC7A11 and GPX4, upregulates ferroptosis-related markers (e.g., ACSL4 and LOX), and thereby induces ferroptosis [70]. To sum up, the p53-SLC7A11 axis serves as a central regulatory hub in ferroptosis, orchestrating multifaceted interplays among redox homeostasis, iron metabolism, and lipid peroxidation. Future research priorities include overcoming resistance bottlenecks, developing targeted combination strategies, and expanding its therapeutic scope to non-oncological disease areas.

3.5. The AMPK/mTOR signaling axis regulates ferroptosis and therapeutic potential in disease

AMP-activated protein kinase (AMPK), a serine/threonine kinase, modulates autophagy via the mTOR pathway and coordinates cellular responses to oxidative stress [71,72]. mTOR (mammalian target of rapamycin), a phosphatidylinositol 3-kinase-related kinase (PIKK), functions as a serine/threonine kinase [73]. Moreover, prior research has demonstrated that SIRT3 drives autophagy-dependent ferroptosis via AMPK-mTOR activation. SIRT3 knockout lowers p-AMPK and activates mTOR, suppressing autophagy markers (LC3B-II/Beclin1) and thereby inhibiting autophagy-dependent ferroptosis [74].

FIN56 (a type III ferroptosis inducer) and the mTOR inhibitor Torin2 synergistically trigger autophagy-dependent ferroptosis in bladder cancer. This synergy operates through dual mechanisms: GPX4 downregulation by FIN56 and Torin2-mediated ULK1-dependent autophagy activation that promotes lipid peroxidation [75]. Bupivacaine triggers ferroptosis in bladder cancer through mTOR dephosphorylation and PI3K/AKT/mTOR pathway suppression. When mTOR is inhibited by bupivacaine, autophagy-related proteins ULK1 and ATG13 undergo dephosphorylation, leading to autophagy activation. This autophagic process mediates the degradation of FTH1 through NCOA4, liberating free Fe^{2+} that fuels lipid peroxidation. Furthermore, mTOR signaling transcriptionally regulates GPX4 via SREBP1. Bupivacaine-mediated mTOR suppression markedly reduces GPX4 levels, consequently enhancing lipid peroxidation and triggering autophagy-dependent ferroptosis [76]. Future research should delineate the interplay between AMPK/mTOR signaling and novel ferroptosis regulators to optimize therapeutic approaches for lipid-rich malignancies and metabolic diseases.

3.6. The JAK-STAT signaling pathway in ferroptosis and disease pathogenesis

The JAK/STAT signaling pathway was first identified in 1989 [77]. It is an intracellular signal transduction pathway that is ubiquitously expressed and plays a crucial role in various vital biological processes, such as immune regulation, cell proliferation, differentiation, and apoptosis [78]. This pathway involves receptor-ligand complexes, Janus kinases (JAKs), signal transducer and activator of transcription (STAT) proteins, as well as the suppressors of cytokine signaling (SOCS) and cytokine-inducible Src homology 2 -containing protein family, all of which are crucial for the precise regulation of JAK/STAT signaling function [79]. Activation occurs when cytokines (e.g., ILs, IFNs, GM-CSF) bind their cognate transmembrane receptors, triggering receptor oligomerization. This facilitates the phosphorylation and activation of associated JAKs, which subsequently phosphorylate STAT proteins. Phosphorylated STATs then dimerize, translocate to the nucleus, and drive the transcription of cytokine-responsive genes [78,80]. The JAK-STAT pathway centrally regulates key physiological and pathological cellular processes [81,82].

IFN-γ enhances tumor ferroptosis sensitivity via JAK-STAT to promote antitumor immunity. In the HCC cells, ferroptosis inducer Erastin suppresses system Xc^-, blocking cystine uptake and depleting intracellular GSH. This triggers lipid peroxidation accumulation while concurrently promoting the release of tumor-associated antigens and exposure of damage-associated molecular patterns (DAMPs). These events activate dendritic cells (DCs) and T cells, stimulating IFN-γ secretion. IFN-γ subsequently activates the JAK/STAT-IRF1 signaling axis, further inhibiting system Xc^- and amplifying lipid peroxidation to suppress xenograft tumor growth. IFN-γ upregulates Acyl-CoA Synthetase Long Chain Family Member 4 (ACSL4), thereby enhancing $CD8^+$ T cell-mediated tumor cell cytotoxicity and establishing an immunometabolic feedforward loop that amplifies ferroptosis [83,84]. These findings suggest that IFN-γ activates JAK-STAT signaling to enhances ferroptosis, potentially providing a mechanism for developing immunotherapeutic strategies in cancer treatment [85].

3.7. The regulatory role of NRF2 in ferroptosis and therapeutic potential in disease

NRF2, encoded by the *NFE2L2* gene, is a pivotal transcription factor in regulating intracellular oxidative stress (OS). It directly modulates the expression of over 200 genes involved in antioxidant responses and phase II detoxification processes. Notably, RF2 regulates ferroptosis via different targets, including those in iron metabolism (FTL, FTH, FPN) and antioxidant defense (GPX4, SLC7A11, GCL, GSH) [86,87]. Under physiological conditions with tightly regulated hydrogen peroxide homeostasis, NRF2 is sequestered in the cytoplasm through its interaction with KEAP1, which targets the transcription factor for ubiquitin-mediated proteasomal degradation [88–90]. After nuclear translocation, NRF2 heterodimerizes with small Maf proteins via its Neh1 domain. This molecular interaction facilitates binding the NRF2-Maf complex to antioxidant response elements (AREs) located in the promoter regions of target genes, thereby initiating their transcriptional activation [86].

Beyond the canonical KEAP1-dependent regulatory mechanism, NRF2 activity is modulated through the PI3K/AKT signaling pathway. This parallel regulatory axis depends on GSK-3β-dependent phosphorylation of NRF2 at critical serine residues, facilitating its recognition by the β-TrCP-CUL1 E3 ubiquitin ligase complex and subsequent proteasomal degradation [91]. Although KEAP1-mediated cytosolic degradation is the primary route for NRF2 turnover, GSK-3β emerges as a key regulator that controls NRF2 stability through phosphorylation [92].

Sipeimine demonstrates significant *anti*-ferroptosis effects by modulating key biochemical markers, including reduced Fe^{2+} levels, malondialdehyde (MDA) content, and pro-inflammatory factors. Sipeimine alleviates PM2.5-induced lung injury via PI3K/AKT activation to upregulate NRF2. Mechanistic studies have revealed that AKT-mediated NRF2 activation plays a crucial regulatory role in ferroptosis suppression during lung injury [93]. Notably, the therapeutic efficacy of sipeimine and rosavin co-administration is significantly attenuated by pretreatment with the specific PI3K inhibitor LY294002. This pharmacological evidence strongly suggests that the combined treatment exerts its *anti*-ferroptosis effects and mitigates PM2.5-induced pulmonary damage primarily via PI3K/AKT/NRF2 activation [94]. NRF2 serves as the central hub in the ferroptosis regulatory network. Its multitarget

nature provides broad-spectrum protection against degenerative diseases and organ damage. Future research should focus on bottlenecks in tissue selectivity, delivery efficiency, and long-term safety. Integrating precision medicine with natural compound-based drug development will facilitate the transition from mechanistic insights to clinical translation.

3.8. Endoplasmic reticulum in ferroptosis regulation and therapeutic implications in disease

The endoplasmic reticulum (ER) serves as the primary site for cellular phospholipid synthesis (e.g., phosphatidylethanolamine, PE). Its membranes are enriched with polyunsaturated fatty acids (PUFAs). Polyunsaturated fatty acids (PUFAs) promote ferroptosis through their intrinsic chemical instability (bis-allylic hydrogen vulnerability), enzymatic incorporation into membrane phospholipids via ACSL4/LPCAT3, and iron-catalyzed peroxidation through Fenton reactions [95]. D-PUFAs (bis-allylic deuterated polyunsaturated fatty acids) refer to structurally modified fatty acids wherein deuterium atoms (D) are site-specifically incorporated at bis-allylic positions of PUFAs via isotopic labeling techniques, thereby conferring significantly augmented antioxidant properties [96]. D-PUFAs introduce deuterium (^{2}H) atoms at bis-allylic sites of polyunsaturated fatty acids (PUFAs), replacing the native hydrogen (^{1}H) atoms. The higher cleavage energy barrier of C-D bonds compared to C–H bonds significantly reduces the rate of hydrogen abstraction by free radicals, thereby disrupting the initiation step of lipid peroxidation chain reactions. Previous studies utilizing stimulated Raman scattering imaging revealed that D-PUFAs could inhibit ferroptosis, which predominantly localize to the ER membrane rather than lipid droplets. Notably, even when lipid droplet accumulation was pharmacologically suppressed, D-PUFAs retained their ability to effectively block ferroptosis, underscoring the ER as the critical site for their protective function [97].

Current pharmacological interventions targeting ER membrane-mediated ferroptosis regulation primarily operate via three mechanisms: inhibiting PUFA-phospholipid biosynthesis, disrupting ER stress signaling pathways, and regulating mitochondria-ER membrane interactions. For instance, ER stress triggers ATF4 activation, which transcriptionally upregulates CHAC1 expression. As a GSH-specific degrading enzyme, CHAC1 catalyzes GSH depletion, thereby compromising GPX4's antioxidant function. This cascade ultimately results in accumulated lipid peroxides that drive ferroptosis execution. Meanwhile, ER stress triggers cytosolic calcium overload, activates the mitochondrial calcium uniporter, leading to collapse of mitochondrial membrane potential and ROS burst, thereby exacerbating ferroptosis [98]. In addition, in diabetic nephropathy, hyperglycemia-induced ER stress activates the XBP1-Hrd1 axis, which suppresses the NRF2 antioxidant pathway and drives ferroptosis in renal tubular cells. Treatment with the ferroptosis inhibitor Ferrostatin-1 blocks this pathway, reducing the urine albumin-to-creatinine ratio by 50 % and ameliorating renal function impairment [99]. This frontier promises to redefine ferroptosis therapeutics, leveraging ER biology to combat diseases where oxidative lipid damage underpins pathology.

3.9. Other regulatory signaling pathways in ferroptosis and therapeutic implications

Thioredoxin 1 (Trx-1), a crucial redox-regulatory protein, plays essential roles in cellular antioxidant defense while modulating transcription factor activity and inhibiting apoptosis [100]. In Parkinson's disease, Trx-1 can impede ferroptosis by modulating GPX4 and GSH [101,102]. In addition to the primary defense mechanisms against ferroptosis mentioned above, other natural antioxidants contribute significantly to protection against ferroptosis. Vitamin E collaborates with GPX4 to uphold lipid redox balance and avert ferroptosis in hematopoietic stem and progenitor cells (HSPCs). Furthermore, its quinone/hydroquinone metabolite assumes responsibility for its protective action against ferroptosis [52,103]. Recent investigations have highlighted the significance of inducible nitric oxide synthase (iNOS)/NO in regulating susceptibility to ferroptosis, particularly in macrophages and microglia [52]. BH4, a potent radical-scavenging antioxidant, attenuates GPX4 inhibitor-induced ferroptosis by suppressing lipid peroxidation [52]. GCH1, the rate-limiting enzyme in BH4 biosynthesis, plays a pivotal role in iron homeostasis and ferroptosis resistance in colorectal cancer. Inhibiting GCH1-mediated BH4 biosynthesis activates ferritinophagy, leading to ferritin degradation and enhanced labile iron release. This synergizes with erastin to amplify ferroptosis, supporting a therapeutic strategy of combining GCH1 inhibitors with erastin. In colorectal cancer cells, GCH1 knockout or treatment with BH4 inhibitors significantly enhances erastin-induced lipid peroxidation and cell death [104].

4. Structure and function of DHODH protein and its defense mechanism against ferroptosis

4.1. Introduction of DHODH and its relationship with cell growth and proliferation

Human DHODH, localized to 16q22 (1191 bp), is a 397-amino acid flavin-dependent iron enzyme mediating the rate-limiting step in pyrimidine synthesis [10,48]. DHODH exhibits distinct subcellular localization patterns across evolutionary lineages, correlating with its functional integration into organism-specific metabolic systems. In prokaryotes, the enzyme (encoded by pyrD) resides on the cytoplasmic membrane's inner face. In the yeast *Saccharomyces cerevisiae*, DHODH is a cytosolic isoform encoded by *URA1*. In higher eukaryotes, DHODH is specifically targeted to mitochondria and anchored on the outer face of the inner mitochondrial membrane, a conserved localization pattern observed in humans. This topology allows direct electron transfer to ubiquinone Q_{10}, effectively coupling its catalytic activity with the respiratory chain's redox reactions [105]. Phylogenetically classified into two major classes based on sequence homology and subcellular localization, DHODH exhibits distinct structural and functional characteristics across species (Table 1). Class 1 DHODHs comprise soluble cytoplasmic enzymes with three subtypes: (1) Class 1A, homodimeric proteins predominant in Gram-positive bacteria. (2) Class 1B, Heterotetramer complexes in Gram-positive species [106]. (3) Class 1S, a novel subtype employing serine as a catalytic base without natural electron acceptors [107,108]. The taxonomic classification and functional role of this DHODH subtype remain controversial and require further structural and biochemical validation. In contrast, Class 2 DHODHs are monomeric membrane-bound enzymes anchored to the outer surface of mitochondrial inner membranes in eukaryotes through transmembrane domains. This membrane association enables functional coupling with the respiratory chain via ubiquinone Q_{10}-mediated electron transfer [108–111]. Notably, human DHODH belongs to Class 2 and demonstrates <20 % sequence identity with Class 1 enzymes [112]. Structural analysis reveals conserved catalytic domains containing FAD-binding sites for dihydroorotate (DHO) oxidation, with distinct membrane-interaction modules in Class 2 enzymes. The Class 1 catalytic core features solvent-exposed active sites, while Class 2 enzymes position their catalytic pocket toward the mitochondrial intermembrane space. This topological arrangement facilitates electron transfer to coenzyme Q in the respiratory chain, contrasting with Class 1's cytoplasmic redox cycling [112]. The membrane integration of Class 2 DHODH via its N-terminal α-helices contributes to its structural stability within mitochondrial supercomplexes. However, the dynamic regulation of pyrimidine synthesis in response to cellular energy status primarily relies on metabolic feedback mechanisms rather than direct conformational changes induced by membrane anchoring. This functional-geographical specialization highlights the evolutionary adaptation of DHODH isoforms to meet distinct metabolic requirements in prokaryotic versus

Table 1
Summary of DHODH.

Class	Form	Electron Acceptor	Localization	Catalytic base	Functional Features	Species distribution
Class 1A	Homodimer	FMN	Cytosol	Cysteine	The de novo pyrimidine biosynthesis pathway regulates cell proliferation	Gram-positive bacteria; *Saccharomyces cerevisiae*
Class 1B	Heterotetramer	NAD^+	Cytosol	Cysteine	Key enzymes in bacterial pyrimidine metabolism	Gram-positive bacteria
Class 1S	Heterodimer	Coenzyme Q, O2	Cytosol	Serine	Its function may be associated with adaptation to extreme environments	*Sulfolobus solfataricus*
Class 2	Monomer	Quinones	Cell membrane or inner mitochondrial membrane	Serine	Drives pyrimidine biosynthesis by oxidizing DHO to orotate via quinone-dependent electron transfer	Gram-negative bacteria; Mammals; *Candida* spp

eukaryotic systems [113].

In the human body, pyrimidine synthesis occurs through the salvage and de novo synthesis pathways (Fig. 2). DHODH is a key participant in the de novo synthesis of pyrimidines [114], which is a six-step pathway essential for nucleotide biosynthesis. The first three steps are catalyzed by the multifunctional CAD enzyme, which integrates three catalytic domains: carbamoyl phosphate synthetase II (CPS II), aspartate transcarbamoylase (ATC), and dihydroorotas. CAD utilizes L-glutamine, aspartate, and bicarbonate to synthesize carbamoyl phosphate, carbamoyl aspartate, and DHO sequentially. In the fourth step, DHODH converts DHO to orotate (ORO) via coenzyme Q-mediated electron transfer to the mitochondrial respiratory chain [115]. ORO is then converted to uridine monophosphate (UMP) through two cytosolic steps: (1) phosphoribosylation by orotate phosphoribosyl transferase (OPRT) using PRPP (phosphoribosyl pyrophosphate) to form orotidine 5′-monophosphate (OMP), followed by (2) decarboxylation catalyzed by OMP decarboxylase to yield UMP. UMP serves as the central precursor for all pyrimidine nucleotides. It is phosphorylated to uridine triphosphate (UTP), which directly participates in RNA synthesis, including ribosomal biogenesis [10,48]. UTP is further aminated to cytidine

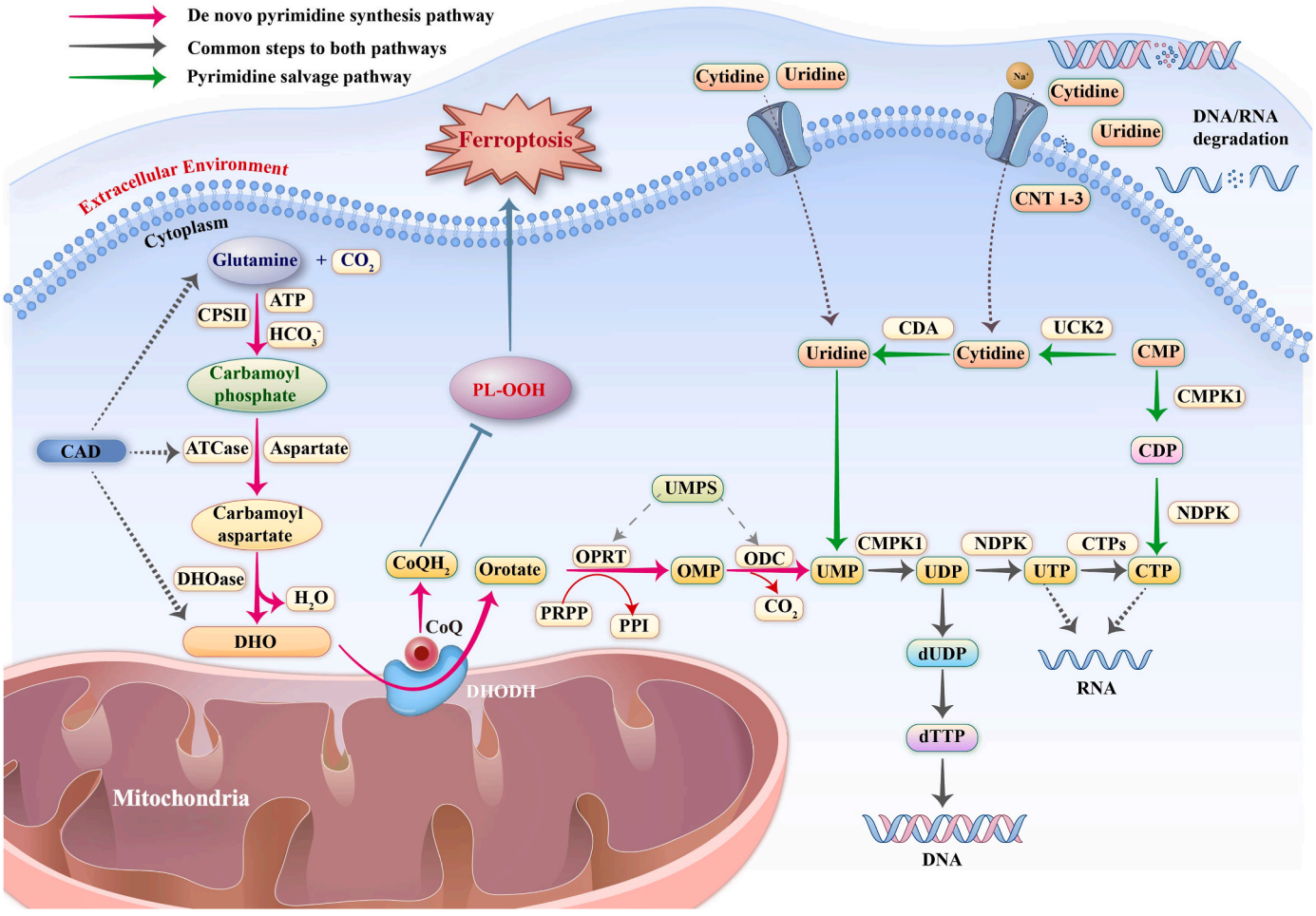

Fig. 2. The pyrimidine synthesis pathway
The de novo pyrimidine biosynthesis pathway (indicated by red arrows) initiates with the CAD enzyme-catalyzed conversion of glutamine, bicarbonate, and aspartate into dihydroorotate. Subsequently, DHODH, located in the inner mitochondrial membrane, mediates the oxidation of dihydroorotate to orotate, which is further processed by the bifunctional UMPS enzyme to yield UMP. The process is accompanied by the production of $CoQH_2$, which can inhibit the generation of mitochondrial lipid peroxides, thereby resisting ferroptosis. In contrast, the pyrimidine salvage pathway (denoted by green arrows) utilizes extracellular uridine and cytidine, which are imported into cells via ENTs and CNTs. These nucleosides are then metabolized into UMP and CTP, respectively. Black arrows represent steps common to both pathways.

triphosphate (CTP), completing the de novo synthesis of major pyrimidine nucleotides. This pathway is rate-limited by CAD and DHODH activities, as their catalytic efficiency dictates the flux of pyrimidine precursors required for DNA/RNA synthesis, thereby governing cellular proliferation and survival. In contrast, Flavin mononucleotide (FMN) is synthesized from riboflavin (vitamin B_2) through ATP-dependent phosphorylation catalyzed by riboflavin kinase. The regeneration of FMN is necessary for continuous DHODH catalysis. CoQ functions as a mitochondrial electron shuttle in the ETC. Complex I's catalytic cycle channels NADH-derived electrons from FMN through iron-sulfur (Fe–S) clusters to ubiquinone. This reduces CoQ to $CoQH_2$. ATP synthase harnesses the proton gradient established by electron transport through the inner mitochondrial membrane to catalyze ATP formation [115,116]. DHODH plays a distinctive role by connecting pyrimidine synthesis with the electron transport chain of aerobic respiration. This connection establishes a vital link between nucleotide metabolism and mitochondrial function, making DHODH an appealing target for drug intervention in pyrimidine synthesis. Inhibiting DHODH pharmacologically has been demonstrated to diminish mitochondrial respiration and stimulate glycolysis. This intricate role of DHODH highlights its significance in coordinating cellular processes and presents potential avenues for therapeutic intervention [116,117].

The specific cell type and developmental phase dictate cellular requirements for pyrimidines. Cells with typical growth rates can acquire adequate pyrimidines through the normal metabolic cycle (salvage pathway) with minimal reliance on the de novo pathway. In contrast, proliferating cells, including cancer and lymphocytes, rely heavily on de novo nucleotide synthesis to meet their heightened demand for nucleotide precursors and biomass components, as the salvage pathway of pyrimidines cannot provide a sufficient supply of nucleosides for their survival [114]. The nucleotide de novo synthesis pathway in which DHODH participates enables cancer cells to generate substrates required for sustained DNA replication without the constraints of the nucleotide **s**alvage pathway. Thus, DHODH, functioning as a flux-limiting enzyme in de novo pyrimidine biosynthesis, is frequently amplified in malignancies to sustain nucleotide pools essential for oncogenic proliferation [118]. Numerous studies have shown that the expression of DHODH is associated with cell growth and proliferation. For instance, overexpression of DHODH in cancer cells can promote cell proliferation, while its inhibition can lead to cell cycle arrest and apoptosis [119]. The proliferation and tumorigenicity of MYCN-expanded neuroblastoma cell lines require high DHODH expression, and DHODH plays an essential role in maintaining the proliferative state of neuroblastoma cells in vitro and in vivo. It was shown that inhibition of DHODH enzyme activity inhibited neuroblastoma cell proliferation [120]. Furthermore, research has found that DHODH activity is upregulated in across multiple cancer types, particularly colorectal, pancreatic, and lung carcinomas, suggesting its potential as a prognostic biomarker and therapeutic target for these malignant tumors [121].

Besides, DHODH is a validated drug target for proliferative diseases, parasitic infections, and malaria due to its essential roles in pyrimidine synthesis and mitochondrial function [9]. DHODH inhibitors have also been used in the treatment of rheumatoid arthritis and multiple sclerosis [48]. Further details will be elaborated in subsequent sections. The development of inhibitors targeting DHODH has provided valuable insights into its biological functions and highlighted its potential as a novel therapeutic target. Further research is needed to define DHODH regulation and pathogenic roles, informing the development of DHODH-targeted therapies.

4.2. The regulation of oxidative stress and energy metabolism by DHODH

Emerging evidence demonstrates that DHODH, while maintaining its canonical role in pyrimidine biosynthesis, exhibits metabolic pleiotropy through its ability to directly modulate mitochondrial metabolic networks and indirectly regulate glycolytic flux. Enhanced DHODH activity depletes the mitochondrial CoQ pool, impairing ETC function and reducing ATP synthesis efficiency. The subsequent decline in the ATP/ADP ratio activates the AMPK signaling pathway. Activated AMPK phosphorylates and upregulates key glycolytic enzymes (e.g., PFKFB3), ultimately promoting glycolysis. Mitochondrial dysfunction (e.g., ETC inhibition caused by DHODH suppression) elevates cytosolic NADH levels and reduces the NAD^+/NADH ratio. As NAD^+ serves as an essential cofactor for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in glycolysis, its depletion directly inhibits GAPDH activity, thereby attenuating glycolytic flux [122,123]. These connections indirectly affect the cell's energy metabolism and redox status [124].

DHODH is a key enzyme that links pyrimidine biosynthesis to the mitochondrial ETC. It transfers electrons from DHO to CoQ, thereby maintaining the normal function of the ETC. Inhibition of DHODH disrupts electron flow, leading to electron accumulation within the ETC, particularly at Complex I and III. This electron leakage results in the reaction of electrons with oxygen, generating superoxide anion [125]. CoQ is an essential component of the ETC and a critical antioxidant. DHODH serves as one of the primary electron donors for CoQ in mitochondria. Inhibition of DHODH reduces the reduced form of CoQ, causing an imbalance in the CoQ pool and further exacerbating ROS accumulation [126]. This, in turn, disrupts the permeability of the mitochondrial inner membrane, leading to a decline in mitochondrial membrane potential and impaired ATP synthesis. Additionally, DHODH inhibition may impair the synthesis of mitochondrial-encoded proteins, such as subunits of the ETC complexes. Severe oxidative stress triggers mitophagy, a selective autophagy process that removes damaged mitochondria [127].

DHODH regulates mitochondrial energy metabolism through the following pathways: (1) DHODH modulates mitochondrial respiration by channeling electrons via its redox activity to CoQ, which subsequently delivers them to the respiratory chain Complex III [128]. Complex III is a key component of the ETC and plays a crucial role in driving ATP production through oxidative phosphorylation [129]. Therefore, the activity of DHODH indirectly regulates ATP synthesis by affecting the electron flow within the respiratory chain, thereby controlling the cell's energy metabolic state. (2) Regulation of mitochondrial membrane potential. Changes in DHODH activity may affect the mitochondrial membrane potential. The membrane potential is an important indicator of intracellular energy production and is directly related to the efficiency of ATP synthesis. When DHODH activity is high, it may influence the mitochondrial membrane potential, thereby affecting the respiratory chain and ATP synthesis processes [130]. Variations in membrane potential can also impact mitochondrial functions, such as ATP synthesis and Ca^{2+} storage. Therefore, the regulation of DHODH may modulate mitochondrial energy metabolism by altering the membrane potential. (3) Regulation of feedback mechanisms in energy metabolism. Elevated energy demand activates sensors (e.g., AMPK, CaMKII), driving oxidative phosphorylation and substrate oxidation to boost ATP production. Conversely, metabolic priorities shift toward anabolism through ATP-driven allosteric control under energy-replete conditions. DHODH epitomizes this metabolic integration by simultaneously driving de novo pyrimidine biosynthesis and consuming mitochondrial CoQ as an electron acceptor, thus directly linking nucleotide production to respiratory chain activity. While DHODH operates without direct allosteric control, its activity is governed through upstream regulation of CAD, which undergoes UTP/CTP-mediated feedback inhibition and AMPK-dependent phosphorylation during energy stress. This dual regulatory mechanism maintains basal pyrimidine flux during nutrient sufficiency while promoting biosynthesis during proliferation or energy crisis to meet nucleic acid demands, creating a metabolite-responsive system that precisely matches nucleotide production with cellular energy status [131]. (4) Regulation of energy metabolism through superoxide anion [132]. The redox process in the DHODH-catalyzed reaction generates a certain amount of electrons, which are transferred through the respiratory chain

complexes and may produce ROS. Excessive ROS can damage mitochondria, inhibit ATP synthesis, and affect cellular energy metabolism. (5) Impact on cellular metabolic state and cell proliferation. The pyrimidine biosynthesis pathway involving DHODH is closely related to processes such as cell proliferation and division. During rapid cell proliferation, the dual demands of pyrimidine nucleotide biosynthesis and increased energy requirements are met through DHODH activity, which directly sustains nucleotide production while concurrently supporting energy metabolism via its coupling to mitochondrial electron transport [125]. (6) Crosstalk with other metabolic pathway. DHODH not only affects energy metabolism itself but also interacts with other metabolic pathway, such as glycolysis. Under certain conditions, DHODH can optimize cellular energy production through its cross-regulation with this metabolic pathway [125]. Glycolysis serves as the primary cytoplasmic route for cellular energy production. However, pyruvate, the end product of glycolysis, enters mitochondria, converting to acetyl-CoA to fuel ATP production via the TCA cycle. DHODH activity modulates mitochondrial redox homeostasis and energy flux, thereby indirectly influencing TCA cycle intermediates such as citrate and α-ketoglutarate, which subsequently regulate glycolysis through metabolic crosstalk. DHODH indirectly affects cellular energy demand through these interactions, thereby influencing glycolysis and subsequent metabolic pathways [133].

Overall, DHODH regulates mitochondrial energy metabolism through various mechanisms. It indirectly modulates ATP synthesis by influencing the respiratory chain, oxidative phosphorylation, and membrane potential. The activity of DHODH may also optimize the mitochondrial energy metabolic state by altering intracellular energy demand, ROS levels, and feedback mechanisms. Its regulatory role in the glycolytic pathway is exerted through multiple indirect mechanisms, and the specific mechanisms still require further investigation.

4.3. DHODH inhibits mitochondrial damage, and its absence or inactivation promotes ferroptosis

As previously described, cells have developed three spatially segregated ferroptosis defense systems, with cytoplasmic and mitochondrial localized GPX4 playing a major protective role against lipid peroxidation, FSP1 acting mainly at the plasma membrane, and DHODH being a major defense arm in mitochondria. DHODH functions as a mitochondria-specific ferroptosis defense system that operates independently of both cytosolic GPX4 and membrane-associated FSP1 [134]. DHODH is a mitochondrial inner membrane enzyme that, as described above, links the pyrimidine biosynthesis pathway to the mitochondrial electron transport chain. Several lines of evidence support the link between the inhibition of DHODH and ferroptosis.

Mao et al. revealed that DHODH suppresses mitochondrial lipid peroxidation by maintaining CoQ redox cycling, whereas FIN56 promotes ferroptosis through GPX4 degradation and CoQ exhaustion, collectively exacerbating mitochondrial oxidative damage [122]. Further investigation revealed that DHODH mediates the conversion of CoQ to $CoQH_2$ within the inner mitochondrial membrane. Supplementation with $mitoQH_2$, a mitochondrial-targeted analog of $CoQH_2$, significantly inhibited the death of DHODH KO cells. $MitoQH_2$ is the reduced form of MitoQ, containing two hydrogen atoms, and can donate electrons more effectively to neutralize free radicals in antioxidant reactions. The reduced form of $MitoQH_2$ generally exhibits more substantial antioxidant capabilities than the oxidized form of MitoQ, as it is more readily involved in redox reactions, thereby being more efficient in reducing ROS [135]. This suggests that $mitoQH_2$ primarily inhibits ferroptosis in cells with mitochondrial lipid peroxidation and that DHODH is required to sustain the reduction of mitoQ to $mitoQH_2$ to suppress ferroptosis [122].

Besides, DHODH depletion triggers mitochondrial lipid peroxidation and ferroptosis in mitoGPX4-deficient tumors. DHODH depletion fails to trigger lipid peroxidation in mitoGPX4-high tumors, but synergizes with ferroptosis inducers to amplify peroxidation and ferroptosis. Moreover, GPX4-low tumor cells showed greater sensitivity to DHODH inhibitors than GPX4-high counterparts. A potential mechanism is that DHODH inhibition upregulates ferroptosis-linked PTGS2 specifically in GPX4-low cells, enhancing lipid peroxidation [122].

Studies have revealed that when GPX4 is inhibited, the function of SLC7A11 becomes constrained, compelling cells to rely on DHODH to suppress lipid peroxidation by maintaining $CoQH_2$ levels, thereby defending against ferroptosis. In cells with low SLC7A11 expression or functional deficiency, DHODH plays an especially critical role, serving as a key backup mechanism to inhibit ferroptosis. DHODH participates in pyrimidine nucleotide synthesis, while SLC7A11 regulates cysteine metabolism and GSH synthesis, maintaining cellular redox balance and metabolic homeostasis. Inhibition of DHODH enhances ferroptosis sensitivity in SLC7A11-deficient cells, highlighting the metabolic interplay between these two pathways. This dual defense mechanism offers novel therapeutic targets for cancer treatment, as combined inhibition of DHODH and SLC7A11 significantly enhances ferroptosis induction, underscoring its profound clinical implications [122].

As previously noted, DHODH catalyzes the rate-limiting step in de novo pyrimidine biosynthesis, and its inhibition can induce purine depletion with reduced synthesis of pyrimidine nucleotides [118]. This metabolic perturbation is particularly consequential given that nucleoside triphosphates (NTPs) which are are derived from purines and pyrimidines serve as essential precursors for deoxynucleoside triphosphate (dNTP) biosynthesis. Ribonucleotide reductase (RNR) mediates the rate-limiting conversion of NTPs to dNTPs, with its activity being downregulated during pyrimidine nucleotide depletion. Thus, DHODH inhibition triggers pyrimidine depletion, impairing RNR activity and depleting dNTP pools. The catalytic function of RNR requires GSH as a cofactor to reduce NDPs to dNDPs. Consequently, impaired RNR activity diminishes GSH consumption during this process, and thereby suppressing ferroptosis. Thus, DHODH depletion could protect against ferroptosis through RNR-dependent GSH recycling and GPX4 activation [125].

Previous studies have demonstrated that DHODH inhibition disrupts pyrimidine nucleotide metabolism, leading to an imbalance in the intracellular nucleotide pool. This metabolic stress triggers the stabilization and accumulation of p53, ultimately enhancing p53-dependent tumor suppressive function. The accumulated p53 activates downstream target genes, inducing cell cycle arrest and ferroptosis. These findings support dual DHODH/p53 inhibition as a metabolically driven tumor suppression strategy [136].

In summary, DHODH inactivation promotes ferroptosis through multiple mechanisms: reducing CoQ_{10} levels, increasing ROS production, and depleting pyrimidine pools, which collectively impair GSH synthesis and enhance lipid peroxidation. Additional investigations are warranted to elucidate the molecular mechanisms underlying DHODH-mediated ferroptosis regulation and to assess its translational applicability in targeting mitochondrial lipid peroxidation pathways.

5. Application of DHODH in disease treatment

5.1. DHODH offers a new targeting strategy for cancer treatment

Recent studies demonstrate that tumor suppressors (e.g., p53, BAP1) promote ferroptosis by inhibiting SLC7A11, thereby restricting cystine uptake and depleting GSH. Cancer cells exhibit heightened susceptibility due to their iron addiction and frequent dysregulation of ferroptosis defenses (e.g., GPX4 loss, SLC7A11 amplification) [137]. This vulnerability is exacerbated by redox imbalance—a core oncogenic feature—where accumulated H_2O_2 fuels proliferation while demanding compensatory antioxidant mechanisms. Mitochondria regulate this balance by supplying biosynthetic intermediates, controlling ferroptosis sensitivity, and acting as major H_2O_2 reservoirs. Importantly, DHODH fine-tunes redox homeostasis and H_2O_2 flux through CoQ pool

modulation, creating a metabolic network that links pyrimidine synthesis to stress adaptation, ultimately supporting tumor growth and ferroptosis evasion [128].

As mentioned above, Proliferating cells prioritize de novo pyrimidine synthesis over salvage pathways to meet replication-driven nucleic acid demands. Thus, DHODH is more critical in cancer and rapidly proliferating cells. Targeting DHODH through inhibitors or RNA interference can induce pyrimidine depletion, effectively inhibiting cell proliferation. In the microenvironment of tumor hypoxia and malnutrition, cancer cells rely heavily on the pyrimidine de novo synthesis pathway and are, therefore, highly sensitive to DHODH inhibitors. Moreover, DHODH inhibition depletes pyrimidine pools (e.g., UTP), impairing ribosome biogenesis to activate p53-mediated cell cycle arrest. All of these suggest that DHODH inhibitors have greater potential in treating malignant tumors [10,138].

For example, the growth of PTEN mutant cells depends on glutamine fluxes in the pyrimidine de novo synthesis pathway and is sensitive to DHODH inhibition. S-phase PTEN mutant cells showed an increased number of replication forks, while DHODH inhibition resulted in the underactivation of ATR and DNA damage to replication forks, chromosome breakage, and cell death. Consequently, replication stalls due to nucleotide insufficiency, selectively impairing PTEN-deficient cell proliferation. This synthetic vulnerability positions DHODH inhibitors as a promising therapeutic strategy for PTEN-mutant cancers [138,139]. DHODH is required for pyrimidine de novo synthesis, and liver kinase B1 (LKB1) -inactivated tumors are susceptible to pyrimidine metabolism disruption; thus, DHODH inhibition has become a therapeutic target for this type of tumor. A771726, the bioactive leflunomide metabolite, acts as a DHODH inhibitor and has demonstrated potent antitumor efficacy against LKB1-deficient malignancies, including suppression of primary tumor growth and distant metastasis [140]. Previous studies showed leflunomide inhibits DHODH, inducing mitochondrial lipid peroxidation. For instance, In HCC chemotherapy-resistant models, leflunomide suppresses DHODH, disrupting mitochondrial ETC function and CoQ regeneration. This impairment leads to ROS accumulation and uncontrolled lipid peroxidation, thereby promoting ferroptosis in HCC cells through iron-dependent phospholipid peroxidation pathways [141]. Furthermore, co-treatment with the DHODH inhibitor brequinar and SLC7A11 inhibitors (e.g., Erastin) synergistically enhances ferroptosis sensitivity [122]. This study also fully demonstrated the inhibitory effect of DHODH inhibitors on cancer and the prospect of their application.

Previous studies have demonstrated that AML cells exhibit heightened sensitivity to DHODH depletion [142]. DHODH inhibition induces pyrimidine starvation, triggering AML blast cell death and differentiation, accompanied by morphological alterations, surface marker changes, and transcriptional reprogramming. Mechanistically, DHODH blockade promotes myeloid differentiation in both human and murine AML models through MYC downregulation, a key transcriptional regulator of tumor cell differentiation [142,143].

DHODH is overexpressed in human breast cancer tissues, with high-DHODH-expressing cells exhibiting enhanced sensitivity to DHODH inhibition. In DHODH inhibitor-sensitive breast cancer models (T-47D and MDA-MB-231), treatment induces metabolic stress through ATP depletion without ROS elevation, accompanied by S-phase cell cycle arrest and upregulation of p53/p65/STAT6 signaling pathways - a response profile distinct from the resistant phenotypes seen in MDA-MB-436 and W3.006 cell lines [10,144]. In another study, CAPE (caffeic acid phenethyl ester), a known DHODH inhibitor, demonstrates broad-spectrum cytotoxicity against multiple breast cancer cell lines, including MCF-7, T-47D, BT-474. Meanwhile, the pyrimidine analog 5-fluorouracil (5-FU)—a mainstay chemotherapeutic agent—exerts its effects through thymidylate synthase inhibition, inducing S-phase arrest and apoptosis. This study reveals that CAPE potentiates 5-FU's anticancer activity by synergistically enhancing both intrinsic and extrinsic apoptotic pathways. Notably, the CAPE/5-FU combination significantly lowers the therapeutic threshold for 5-FU in breast cancer models while reducing the likelihood of dose-limiting toxicities [145]. Although extensive specialized research has been conducted on breast cancer, studies investigating the regulatory role of DHODH inhibitors in promoting ferroptosis in breast cancer cells remain limited. Future efforts should focus on elucidating the interactive mechanisms between DHODH and breast cancer-specific metabolic reprogramming, developing mitochondrial-targeted DHODH inhibitors to enhance tumor selectivity, and exploring synergistic strategies with iron chelators, antioxidants, or immune checkpoint inhibitors.

Analysis of the Oncomine dataset reveals significantly elevated DHODH expression in colorectal cancer (CRC) tumor tissues compared to normal samples. DHODH overexpression promotes CRC cell proliferation, and its inhibition exerts anti-proliferative effects, with sensitivity to DHODH inhibitors being influenced by cellular gene expression profiles [105]. DHODH inhibitors synergistically drive ferroptosis in CRC cells through multiple pathways. DHODH and FSP1 on the plasma membrane together constitute a dual defense system for CoQ reduction. DHODH inhibitors, by suppressing mitochondrial $CoQH_2$ generation, force cells to rely on FSP1 to maintain antioxidant capacity. In turn, FSP1 inhibitors (e.g., iFSP1) can further block this compensatory mechanism, establishing a synergistic lethal effect. Previous experiments have demonstrated that the combination of DHODH inhibitors and FSP1 inhibitors significantly induces ferroptosis in CRC cells [146]. Given that DHODH inhibitors have entered clinical trials, they may emerge as a promising precision therapy for CRC.

DHODH is upregulated in skin cancers, including actinic keratoses, Bowen's disease, and squamous cell carcinoma, with activity levels approximately double those in normal skin [147]. Mechanistically, DHODH drives UVB-induced energy metabolism reprogramming through STAT3-mediated transcriptional activation, and its inhibition blocks keratinocyte neoplastic transformation [148]. In melanoma, DHODH inhibition disrupts transcriptional elongation of key oncogenes (e.g., Myc) and induces cell cycle arrest through p53 activation [136]. Preclinical studies demonstrate that DHODH inhibitors (e.g., leflunomide, BRQ, HZ-05) effectively suppress skin cancer progression by targeting multiple pathways, highlighting their therapeutic potential in nonmelanoma skin cancers and melanoma [149]. In melanoma B16F10 and A375 cells, the DHODH inhibitor B2 blocks the ability of DHODH to reduce CoQ to $CoQH_2$, leading to a decline in mitochondrial antioxidant capacity and a significant increase in the lipid peroxidation marker MDA. Additionally, B2 inhibits methylenetetrahydrofolate dehydrogenase 2, reduces programmed death-ligand 1 expression, alleviates immune suppression, and indirectly enhances the ferroptotic effect [150]. Given the characteristic metabolic profile of skin cancers - including pyrimidine dependency and heterogeneous GPX4 expression - pharmacological targeting of DHODH emerges as a promising therapeutic approach.

Recent studies have highlighted the therapeutic potential of DHODH inhibition across various cancer types through distinct mechanisms. In cervical cancer, DHODH blockade inhibits proliferation and enhances cisplatin-induced ferroptosis through mTOR suppression, showing robust anti-tumor activity in vitro and in vivo [137]. Likewise, in HCC, pairing low-dose oxaliplatin with DHODH inhibitor leflunomide boosts chemosensitivity and lowers toxicity, emerging as a viable strategy for advanced cases [141]. In hepatocellular carcinoma cells with low GPX4 expression, DHODH inhibitors significantly enhance ferroptosis sensitivity. For instance, GPX4-knockout HCC cells exhibit significant sensitivity to DHODH inhibitors (e.g., Brequinar), which can be blocked by ferroptosis inhibitors (e.g., liproxstatin-1) [134].

The role of DHODH in chemoresistance has been particularly noted in gastric cancer, where its upregulation promotes drug resistance through accelerated glycolysis. Inhibition of DHODH sensitizes gastric cancer cells to chemotherapeutic agents, with combination therapies (e.g., leflunomide with 5-FU, DTX, or OXA) showing enhanced apoptosis induction and tumor growth suppression [151]. This chemosensitization effect extends to MYCN-amplified neuroblastoma, where DHODH

inhibition targets pyrimidine nucleotide synthesis. However, the efficacy of DHODH inhibitors as monotherapy is influenced by serum uridine levels, as serum uridine provides pyrimidine nucleotides via the salvage pathway, bypassing the DHODH-mediated de novo synthesis pathway and thereby attenuating the anti-tumor effect. This limitation can be overcome by combining DHODH inhibitors with dipyridamole, an FDA-approved nucleoside transport inhibitor, which synergistically inhibits tumor growth by blocking the compensatory salvage pathway and enforcing reliance on the disrupted de novo synthesis pathway [120]. At high concentrations, DHODH inhibitors such as brequinar predominantly target FSP1, a crucial mitochondrial enzyme that limits lipid peroxidation via CoQ_{10} reduction [146]. By inhibiting FSP1, these compounds exacerbate mitochondrial lipid peroxidation and drive ferroptosis. This mechanism suggests that DHODH inhibitors could similarly increase ferroptosis vulnerability in gastric cancer cells, especially those with intrinsically low GPX4 expression.

DHODH inhibition demonstrates broad anti-tumor activity across multiple cancer types, including multiple myeloma [132], neuroblastoma [152], renal cell carcinoma [153], cervical cancer [154], and glioblastoma [155]. Mechanistically, DHODH blockade induces G1/S arrest and apoptosis through WNT/β-catenin and PI3K/AKT/mTOR pathway modulation [156]. DHODH is consistently overexpressed in malignant versus normal tissues, with elevated levels correlating with advanced glioma grade and poor prognosis [155]. In glioblastoma, DHODH targeting disrupts glioma stem cell maintenance via pyrimidine pool depletion [157]. Lung cancers show similar DHODH upregulation, with BRQ-mediated inhibition suppressing SCLC growth through ferroptosis induction [158]. Pancreatic tumors demonstrate DHODH-driven pyrimidine biosynthesis, where its inhibition causes S-phase arrest and mitochondrial dysfunction in PDAC models [121,159]. Notably, 38 % of endometrial cancers (EC) exhibit DHODH overexpression linked to genomic instability. DHODH inhibition induces replication stress and DNA damage, particularly in high-grade EC, with synergistic effects observed when combined with PARP1 inhibitors-suggesting a precision strategy for aggressive endometrial tumors [160].

Given its broad anticancer potential, DHODH represents an emerging therapeutic target, with combination strategies showing particular promise in recent studies. Preclinical studies support DHODH inhibitors as a potential cancer therapy, particularly in combination with chemotherapy or radiotherapy. Further mechanistic investigations, comprehensive safety and efficacy assessments, and clinical protocol optimization remain essential prior to clinical translation.

5.2. DHODH as a target for the development of antiviral drugs

The development of broad-spectrum antiviral therapeutics represents a critical frontier in pandemic preparedness, offering protection against both emerging and re-emerging viral threats. Host-targeted antiviral agents (HTAs) represent a promising therapeutic strategy by disrupting host factors essential for viral replication. Their broad-spectrum activity, high barrier to resistance, and translational potential make HTAs an attractive avenue for antiviral development. Host DHODH serves as a critical dependency for replication in numerous acute infectious viruses [115]. Emerging evidence highlights DHODH as a novel antiviral drug target. This enzyme plays a key role in the pyrimidine biosynthesis pathway, which is required for DNA and RNA synthesis by host cells and viruses.

DHODH inhibition depletes intracellular pyrimidine pools, thereby impairing viral replication and transmission [9,161]. As previously noted, DHODH inhibitors exhibit selective activity in rapidly proliferating cells while sparing normal cell populations. Substantial evidence demonstrates their efficacy against multiple viral families, including influenza viruses, hepatitis C virus, and human cytomegalovirus. These findings position DHODH as a prime target for developing broad-spectrum antivirals effective against negative-sense RNA viruses, positive-sense RNA viruses, DNA viruses, and retroviruses [9].

In previous studies, various DHODH inhibitors, such as leflunomide, teriflunomide, brequinar, thiazole derivatives S312 and S416, emvododstat (PTC299), vidofludimus calcium (IMU-838), A3, FA-613, BAY2402234, MEDS433, RYL-634, GSK 983, and AR-12 derivatives, have demonstrated strong activity against diverse RNA/DNA viruses. These compounds primarily exert their antiviral effects through DHODH pathway inhibition, which disrupts de novo pyrimidine biosynthesis and consequently suppresses viral replication. Clinically relevant DHODH inhibitors such as leflunomide, brequinar, and S312 demonstrate potent *anti*-SARS-CoV-2 activity both in vitro and in clinical settings, significantly reducing viral shedding duration and inflammatory responses [162]. Supporting these findings, Xiong et al. provided direct evidence of DHODH inhibitors' efficacy against SARS-CoV-2 infection [163]. Leflunomide and its active metabolite teriflunomide - FDA-approved DHODH inhibitors - show promise as potential COVID-19 therapeutics by accelerating viral clearance. However, these preliminary findings require validation through large-scale clinical trials [164]. Terri L. Morton et al. demonstrated that emvododstat effectively hampers the replication of SARS-CoV-2 by impeding the activity of DHODH. This compound functions as a potent dual-action inhibitor, effectively suppressing both immunomodulatory pathways and inflammatory responses. Emvododstat effectively hinders SARS-CoV-2 infection in vivo and decreases virus titers in vitro cell experiments. Furthermore, DHODH inhibition blocks mitochondrial CoQ reduction to $CoQH_2$. This leads to the accumulation of mitochondrial lipid peroxides, ultimately triggering ferroptosis in virus-infected cells (Fig. 3). DHODH inhibitors represent a promising dual-target therapeutic strategy for COVID-19 by simultaneously suppressing viral replication and mitigating hyperinflammation [161]. Ongoing clinical investigations of FDA-approved agents (e.g., leflunomide, brequinar) are evaluating their therapeutic potential, though larger validation studies remain essential to confirm these preliminary findings [162,164].

Anna L et al. identified MEDS433, a human DHODH inhibitor, as a potent suppressor of HSV-1/HSV-2 replication in vitro through specific targeting of DHODH enzymatic activity. This inhibition disrupts viral genome accumulation and late gene expression. Crucially, MEDS433 demonstrates marked synergy with both acyclovir (a standard antiviral) and dipyridamole (a pyrimidine salvage pathway inhibitor) [165]. DHODH inhibitors like MEDS433 and P12-34, which show favorable toxicity profiles and robust antiviral activity, represent promising candidates for clinical development against viral infections, including potential flavivirus outbreaks [162,166].

Although the antiviral mechanism of DHODH inhibitors does not involve ferroptosis, they exert broad-spectrum antiviral effects by blocking pyrimidine nucleotide synthesis (thereby inhibiting the raw materials required for viral replication). Future research should focus on optimizing their pharmacokinetics, minimizing off-target effects, and exploring combinatorial regimens with direct-acting antivirals to enhance efficacy against emerging and resistant viral strains.

5.3. DHODH is an important target for the treatment of autoimmune and inflammatory diseases

Autoimmune disorders develop when immune tolerance mechanisms fail, leading to aberrant immune activation against self-antigens. This breakdown in immunological homeostasis stems from intricate gene-environment interactions that dysregulate adaptive immune components - particularly T and B lymphocytes - culminating in chronic, self-perpetuating inflammatory responses that mediate tissue and organ damage [167]. The pyrimidine biosynthesis pathway plays a pivotal role in immune cell activation and proliferation, with DHODH serving as an essential regulatory enzyme. In rapidly dividing cells, like activated T lymphocytes, elevated de novo pyrimidine biosynthesis markedly amplifies cell growth capacity, bestowing upon them superior growth potential [168]. DHODH inhibition disrupts mitochondrial electron

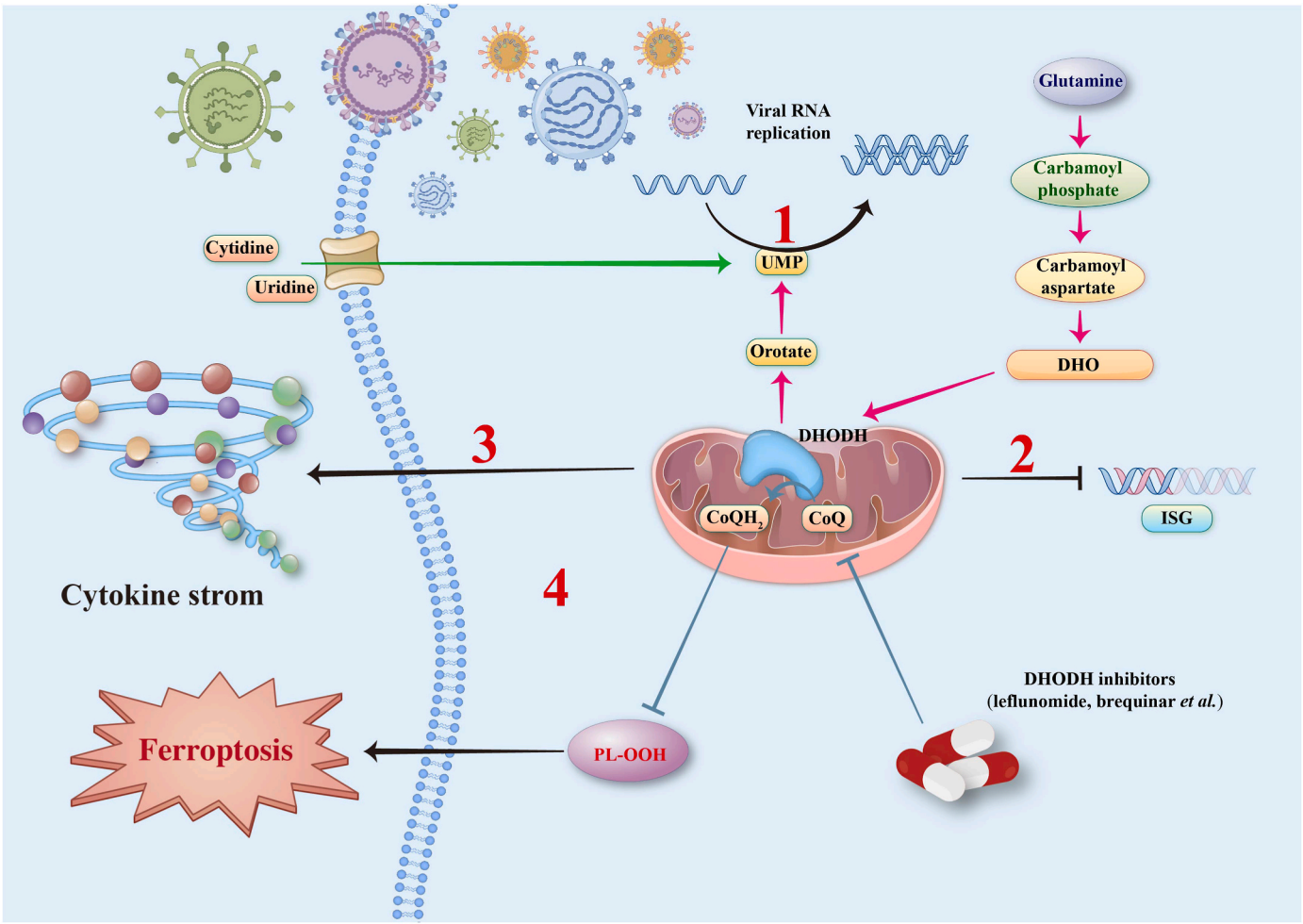

Fig. 3. The role of DHODH inhibitors in viral infection
The quadruple mechanism of DHODH inhibitors encompasses (1) depletion of the pyrimidine nucleotide pool essential for viral replication, (2) activation of interferon-stimulated genes expression, (3) suppression of virus-induced cytokine storm, and (4) Disruption of the CoQ/CoQH2 redox cycle in mitochondria induces ferroptosis via lipid peroxide accumulation. Human cells acquire pyrimidines through two major pathways: de novo biosynthesis (red arrow) and the salvage pathway (green arrow).

transport and de novo pyrimidine synthesis, indirectly affecting oxidative phosphorylation and aerobic glycolysis in activated T cells. This impedes immune cell proliferation, offering a therapeutic strategy for autoimmune diseases such as rheumatoid arthritis and multiple sclerosis (MS) [169]. Human DHODH is recognized as a pivotal target in treating hyperproliferative and inflammatory conditions. Clinically approved DHODH inhibitors, including leflunomide and its active metabolite teriflunomide, are currently used for the treatment of rheumatoid arthritis (RA) and multiple sclerosis (MS) [170,171].

Leflunomide exerts its immunomodulatory effects primarily through DHODH inhibition, thereby blocking de novo pyrimidine biosynthesis. This metabolic constraint selectively impairs the differentiation of naive T cells (T_{Naive}) into effector T cells (T_{eff}), a transition that demands abundant pyrimidine nucleotides to fuel clonal expansion and sustain effector transcriptional programs. Dependence solely on the salvage pathway proves insufficient to meet the biosynthetic demands of effector Teff differentiation, leading to diminished inflammatory cytokine production, a mechanism that offers a viable therapeutic approach for autoimmune disorders [172](Fig. 4). This mechanism underlines leflunomide's effectiveness in autoimmune disease treatment. The active metabolite teriflunomide has demonstrated particular efficacy in MS therapy, limiting CNS infiltration by autoreactive T and B cells [173]. Its established clinical benefits have positioned it as a first-line disease-modifying therapy (DMT) for relapsing-remitting MS (RRMS) [170]. However, therapeutic application requires careful consideration of potential hepatotoxicity and teratogenicity observed in both clinical and preclinical settings.

DHODH inhibitors have been clinically used for rheumatoid arthritis (RA) treatment. These agents exert therapeutic effects by suppressing DHODH, thereby inhibiting the proliferation of activated lymphocytes. Emerging evidence suggests that ferroptosis may contribute to RA pathogenesis, as exemplified by enhanced lipid peroxidation in synovial cells that exacerbate inflammatory responses [174]. Mechanistically, DHODH inhibitors may modulate the mitochondrial CoQ reduction pathway, potentially altering immune cells' susceptibility to ferroptosis and consequently attenuating inflammatory cascades.

Recent advances have uncovered novel DHODH inhibitors featuring enhanced safety characteristics. Notably, Liu et al. demonstrated that piperine, a black pepper-derived bioactive compound, potently inhibits DHODH in T cells. In myelin oligodendrocyte glycoprotein -induced experimental allergic encephalomyelitis models, piperine exhibited robust preventive and therapeutic effects while preserving myelin sheath integrity and blood-brain barrier function. These findings suggest piperine may represent promising next-generation therapeutics for MS with potentially reduced adverse effects [168].

Ferroptosis has been demonstrated to modulates dendritic cell and macrophage activation in autoimmune diseases. The induction of ferroptosis may alleviate inflammatory responses by eliminating

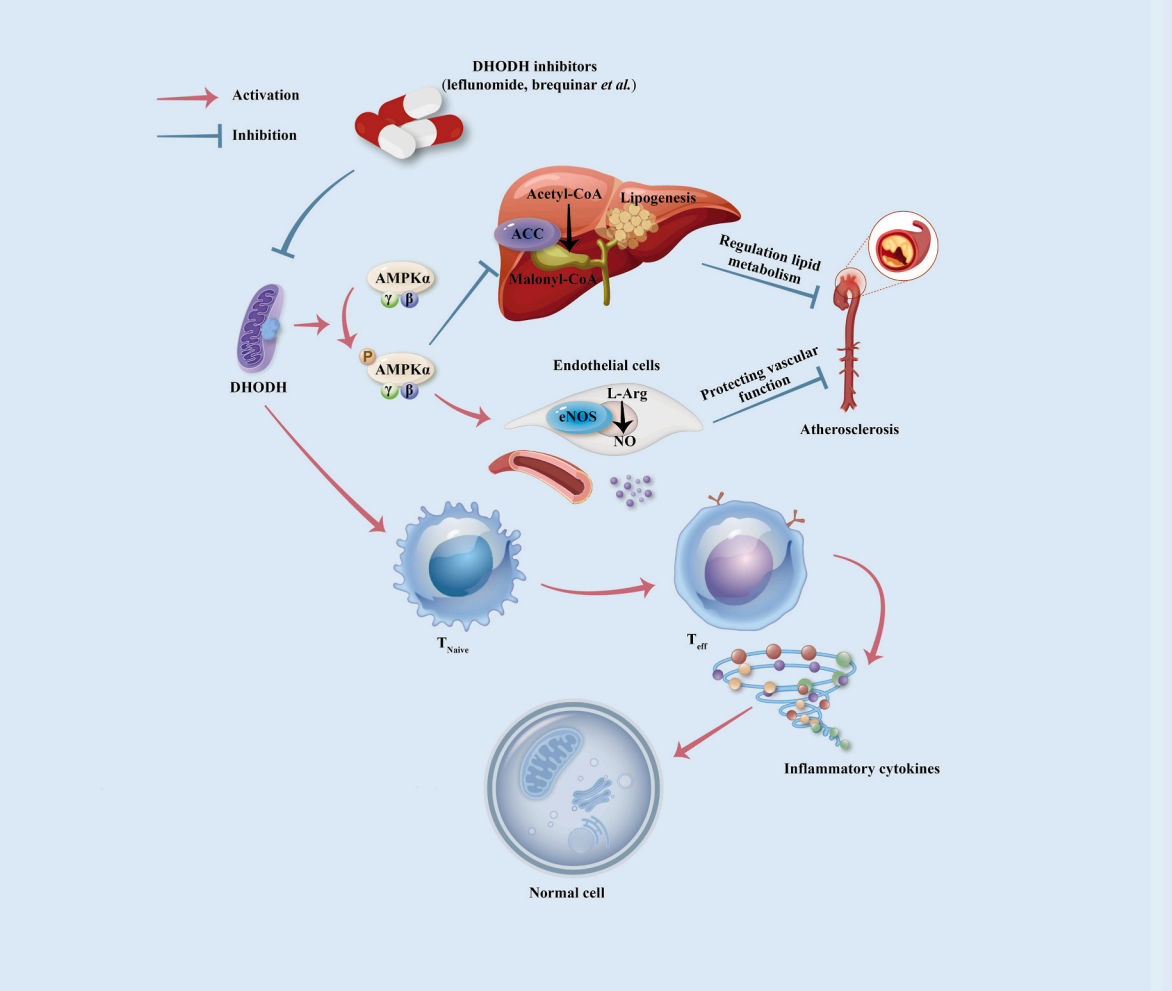

Fig. 4. DHODH inhibitors regulate metabolism, endothelial function, and immune responses
DHODH inhibitors attenuate FFA-induced lipid accumulation in hepatocytes by activating AMPK signaling and suppressing the DHODH pathway, which promotes eNOS phosphorylation to enhance NO production and improve endothelial function. Additionally, DHODH inhibition disrupts de novo pyrimidine synthesis, preventing T_{Naive} from differentiating into T_{eff} and reducing the release of inflammatory cytokines.

hyperactivated immune cells. In certain autoimmune conditions, immune cell survival depends on antioxidant pathways mediated by GPX4 and FSP1 [175,176]. Notably, DHODH inhibitors may synergistically enhance ferroptosis susceptibility by impairing mitochondrial $CoQH_2$ generation, potentially offering a therapeutic strategy for immune modulation.

Prior investigations have demonstrated the noteworthy immunosuppressive and anti-proliferative capabilities of BRQ and leflunomide in T cells. Additionally, DHODH inhibition has the potential to exert anti-inflammatory effects by impeding the generation of pro-inflammatory Th1 effectors and facilitating Th2 cell differentiation in the context of autoimmune disease treatment [10]. In II/III RA trials, leflunomide improved both primary and secondary outcomes, accompanied by a commendable safety profile [171].

Uveitis stands as a T cell-mediated intraocular inflammatory disease and is the foremost cause of blindness in industrialized nations. Given the current approvals limited to cyclosporine A and TNF-α blockers for noninfectious uveitis, there is a significant demand for innovative immunomodulatory and steroid-sparing therapies. Enter KO-100 (PP-001), a novel small molecule inhibitor of DHODH, demonstrating remarkable potency against both T and B cells. In an experimental rat model, KO-100 exhibited high efficacy in treating uveitis. Phase I clinical trial data demonstrated the therapeutic potential of this DHODH inhibitor, with intravitreal administration achieving both intraocular inflammation reduction and significant visual acuity improvement. Critically, the treatment exhibited an excellent safety profile, with no observed ocular toxicity or adverse events. Notably, post-therapeutic resolution of macular edema, a vision-threatening complication, was observed in uveitis patients. These compelling efficacy results position this agent as a promising novel therapy for intraocular inflammatory disease [177].

DHODH inhibitors exert therapeutic effects in autoimmune diseases by inhibiting immune cell proliferation and attenuating inflammatory responses, potentially through ferroptosis induction. While clinical validation is still needed, preclinical evidence indicates these compounds may influence ferroptosis via mitochondrial antioxidant regulation and lipid peroxidation modulation. Additional studies are required to fully characterize their molecular mechanisms across various autoimmune pathologies.

5.4. *DHODH as a therapeutic target for other diseases*

Malaria, a major global health threat caused by *Plasmodium* parasites transmitted through Anopheles mosquito vectors, has been the focus of recent therapeutic strategies targeting essential parasitic metabolic pathways. As *Plasmodium* species exclusively rely on de novo pyrimidine biosynthesis due to their lack of salvage pathway enzymes, DHODH - which mediates both mitochondrial electron transport and the rate-

limiting step in pyrimidine production - emerges as an ideal drug target. The enzyme's critical position in the terminal stage of this indispensable biosynthetic pathway suggests that DHODH inhibition could achieve complete parasitic clearance, offering potential for a definitive malaria cure [178].

Several DHODH inhibitors have been developed as potential antimalarial agents. Among these, inhibitors based on the triazolopyrimidine core scaffold have demonstrated promising bioavailability. Notably, these compounds exhibit high species selectivity for *Plasmodium* falciparum DHODH (PfDHODH), with minimal activity against closely related species [178]. Clinical studies have confirmed that the PfDHODH inhibitor DSM265 is effective against P. falciparum malaria following a single-dose regimen. Its prolonged pharmacodynamic effect suggests potential for preventing disease recurrence after a single administration [179]. Targeting PfDHODH has emerged as a selective antimalarial strategy with low host toxicity. The development of novel, highly specific PfDHODH inhibitors is crucial for combating the rapid emergence of antimalarial drug resistance in *Plasmodium*. This innovative approach holds promise for generating potent antimalarial therapeutics, representing a significant advancement in malaria treatment [180].

Genetic analyses have identified shared non-causal genetic architectures between Alzheimer's disease (AD) and immune-related disorders. Among the genes located at the intersection of AD and immune-mediated diseases, DHODH emerges as a particularly noteworthy protein-coding gene. Consequently, DHODH represents a promising therapeutic target for drug development aimed at simultaneously addressing AD and immune-mediated diseases, as well as their associated complications. This finding highlights the potential for exploring novel therapeutic strategies in this context [181].

Previous studies have demonstrated that leflunomide significantly reduces atherosclerotic plaque area in the aortic arch and root of Western diet-fed ApoE$^{-/-}$ mice by activating the AMPK signaling pathway. Simultaneously, it lowers plasma total cholesterol and triglyceride levels while attenuating hepatic lipid accumulation. Furthermore, leflunomide enhances endothelial function by promoting eNOS phosphorylation via AMPK activation, thereby increasing NO production (Fig. 4) [182].

Genetic studies have established the essential role of *Acinetobacter baumannii* DHODH (AbDHODH) in bacterial survival during rodent infections. Thomas A. Russo and colleagues demonstrated that DSM161—a long half-life AbDHODH inhibitor—provided robust protection in a neutropenic murine thigh infection model. Notably, neither in vitro nor in vivo resistance emerged against DSM161 or related compounds. These findings strongly support the development of AbDHODH-targeted antimicrobials as promising therapeutics for *A. baumannii* and other high-priority bacterial infections [183]. Collectively, DHODH inhibitors represent a dual-purpose therapeutic strategy, offering both antimicrobial efficacy against pathogenic bacteria and immunomodulatory benefits in autoimmune disorders.

Additionally, DHODH inhibitors have been investigated as potential therapeutic agents for parasitic and fungal infections, including schistosomiasis, Phytophthora infestans, and invasive fungal diseases [10]. The development of novel DHODH inhibitors and the optimization of existing compounds may yield more effective treatments for autoimmune and inflammatory disorders (Table 2).

Current research most clearly establishes DHODH inhibition-induced ferroptosis in cancer models, particularly in metastatic or therapy-resistant malignancies. The underlying mechanism involves disruption of the mitochondrial lipid peroxidation defense system, and DHODH inhibitors have demonstrated therapeutic potential in clinical trials. Future studies should explore the applicability of this mechanism across other disease contexts.

Table 2
Drugs targeting the DHODH.

Drug	Disease	Mechanism	Impact	Reference
leflunomide	Skin cancer/HCC	DHODH inhibitors	Exerts tumor-suppressive effects	[141] [149]
CAPE	Breast cancer	DHODH inhibitors	Exerts tumor-suppressive effects and significantly reduces the required concentration of 5-FU	[145]
brequinar	SARS-CoV-2	DHODH inhibitors	Inhibit viral genome replication and subgenomic RNA transcription	[162]
MEDS 433	HSV-1/HSV-2 infection	DHODH inhibitors	Effectively inhibits the replication of HSV-1 and HSV-2	[165]
piperine	Experimental Autoimmune Encephalomyelitis	DHODH inhibitors	Targets DHODH in T cells to exert anti-inflammatory effects	[168]
KO-100	Uveitis	DHODH inhibitors	Alleviates uveitis	[177]
DSM161	*Acinetobacter baumannii* infection	DHODH inhibitors	Inhibits the growth of Acinetobacter baumannii	[183]
DSM265	*P. falciparum* infection	DHODH inhibitors	Inhibits *P. falciparum* infection	[179]

5.5. Summary of clinical trials involving DHODH

Building on DHODH's validated roles in oncogenic proliferation, autoimmune dysregulation, and viral replication pathways, this section evaluates its therapeutic inhibition through an evidence-based lens, synthesizing key clinical trial outcomes. These studies investigate DHODH inhibitors across multiple therapeutic applications, encompassing diverse disease treatments, research methodologies, and clinical development phases. Evidence demonstrates DHODH inhibitors' clinical efficacy and research advancements, underscoring their therapeutic potential and future applications (Table 3).

6. Conclusions and prospects

Ferroptosis maintains a delicate balance between cellular iron homeostasis and oxidative stress, which involves various molecular mechanisms and metabolic pathways. Metabolic interactions and regulatory mechanisms modulate cellular susceptibility to ferroptosis. DHODH contributes to ferroptosis regulation through multiple mechanisms, primarily by depleting mitochondrial CoQ_{10} levels, enhancing ROS generation, and accelerating lipid peroxidation accumulation. Moreover, DHODH plays an important role in the de novo pyrimidine synthesis pathway and connects this pathway to the electron transport chain of aerobic respiration, which makes it the most attractive drug target in the pyrimidine synthesis pathway.

Rapidly developing DHODH inhibitors have demonstrated dual antitumor mechanisms, potentiating cytotoxicity with conventional therapies and suppressing proliferation in preclinical models. Combining DHODH inhibitors with conventional chemotherapy or radiotherapy may be a promising anticancer strategy. DHODH has also emerged as a novel target for antiviral agents and has been suggested to

Table 3
Summary of clinical trials involving DHODH.

Disease	Medicine	Strategy	Phase	NCT
COVID-19 Infection	Brequinar	DHODH inhibitors	II	NCT04575038
COVID-19 Infection	Brequinar	DHODH inhibitors	II	NCT05166876
COVID-19 Infection	Brequinar	DHODH inhibitors	I/II	NCT04425252
SMM	Leflunomide	Suppressing pyrimidine synthesis through Inhibiting DHODH	II	NCT05014646
Autoimmune Diseases	Leflunomide	Suppressing pyrimidine synthesis through Inhibiting DHODH	–	NCT05789017
Henoch-Schonlein Purpura	Leflunomide	Suppressing pyrimidine synthesis through Inhibiting DHODH	–	NCT05937880
MEN1 Gene Mutation	Leflunomide	Suppressing pyrimidine synthesis through Inhibiting DHODH	–	NCT05605587
Primary Sjögren's Syndrome	Leflunomide	Suppressing pyrimidine synthesis through Inhibiting DHODH	II	NCT05113004
Acute Graft Versus Host Disease/ Hematopoietic and Lymphoid System Neoplasm	Leflunomide	Suppressing pyrimidine synthesis through Inhibiting DHODH	I	NCT05443425
PTEN-null Advanced Solid Tumors	Leflunomide	Suppressing pyrimidine synthesis through Inhibiting DHODH	I	NCT04997993
Idiopathic Pulmonary Hemosiderosis/ Leflunomide	Leflunomide	Suppressing pyrimidine synthesis through Inhibiting DHODH	I/II	NCT05937191
Glioma/IDH Mutation	BAY-2402234	Selective DHODH inhibitors	I	NCT05061251
Leukemia	BAY-2402234	Selective DHODH inhibitors	I	NCT03404762
Alopecia Areata	Farudodstat	DHODH inhibitors	II	NCT05865041
Primary Sclerosing Cholangitis	Vidofludimus	DHODH inhibitors	II	NCT03722576
Lymphoma	AG-636	DHODH inhibitors	I	NCT03834584

be of potential benefit for the treatment of proliferative and inflammatory diseases, as well as for other diseases such as malaria.

In the past decades, our understanding of the role of DHODH in the development and pathogenesis of ferroptosis has significantly advanced, and its biological functions in both neoplastic and non-neoplastic diseases have been revealed. Despite these advances, key challenges persist; further studies must elucidate underlying mechanisms, assess safety and efficacy, and optimize clinical protocols before DHODH-mediated ferroptosis modulation can be translated into clinical practice as a broad-spectrum therapeutic strategy.

Here are some challenges: Recent studies have shown that DHODH inhibitors are more effective in malignant tumors with specific genetic backgrounds, such as breast cancer with PTEN loss, melanoma with BRAF (V600E) mutation, and pancreatic cancer with KRAS mutation. Therefore, DHODH inhibitors can be further investigated for targeted therapy with specific genetic backgrounds [10]. While current preclinical studies have primarily focused on solid tumors, emerging evidence suggests that DHODH inhibition may also hold therapeutic potential for non-solid malignancies, warranting further investigation [118]. Future research should aim to (1) elucidate the precise molecular mechanisms by which DHODH modulates ferroptosis; (2) explore potential novel functions of DHODH in ferroptosis regulation beyond its established roles; (3) investigate potential crosstalk between DHODH-mediated pathways and other cellular mechanisms governing lipid peroxidation and iron metabolism. These investigations will provide critical insights for developing DHODH-targeted therapies across diverse cancer types.

DHODH's non-canonical functions beyond mitochondrial metabolism remain unclear. Emerging evidence suggests that DHODH may localize to additional subcellular compartments, including the cytoplasm and membrane-associated structures, where it could potentially regulate distinct cellular processes such as metabolic reprogramming, signal transduction cascades, and stress adaptation mechanisms. Systematic investigation of DHODH's compartment-specific localization patterns and their corresponding functional implications would significantly advance our understanding of its multifaceted roles in cellular homeostasis and disease progression, particularly in the context of cancer metabolism and therapeutic targeting. In addition, emerging evidence underscores the need to advance next-gen DHODH inhibitors with enhanced selectivity for synergistic regimens in iron-metabolism-linked pathologies. However, the potential off-target effects and toxicity of different doses of these inhibitors should not be neglected to avoid limiting their clinical utility.

CRediT authorship contribution statement

Jinghao Cao: Writing – review & editing, Writing – original draft. **Xi Chen:** Writing – review & editing, Writing – original draft. **Lulu Chen:** Writing – original draft, Resources, Data curation. **Yajuan Lu:** Software. **Yunyi Wu:** Visualization. **Aoli Deng:** Resources. **Feifan Pan:** Resources. **Hangqi Huang:** Investigation. **Yingchao Liu:** Resources. **Yanchun Li:** Writing – review & editing, Resources, Conceptualization. **Xiangmin Tong:** Writing – review & editing, Writing – original draft, Resources. **Jing Du:** Writing – review & editing, Writing – original draft, Software, Resources, Funding acquisition.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

Not applicable.

Consent for publication

All authors agree to publish.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

STEAP3, six-transmembrane epithelial antigen of prostate 3; DMT1, divalent metal transporter 1; TFR, transferrin receptor; IRPs, iron regulatory proteins; IRE, iron response element; FPN, ferroprotein; LIP, labile iron pool; GLS, glutaminase; FSP1, ferroptosis inhibitory protein 1; DHO, dihydroorotate; DHODH, dihydroorotate dehydrogenase; $CoQH_2$, dihydroubiquione; SAH, S-adenosylhomocysteine; SAAH, S-adenosine homocysteine hydrolase; HCY, homocysteine; TF, transferrin; GCL, glutamate cysteine ligase; GSS, glutathione synthase; PUFAs, polyunsaturated fatty acids; PL-OOH, lipid hydrogen peroxide; CPSII, Carbamoyl Phosphate Synthetase II; ATCase, Aspartate Trans-Carbamoylase; DHOase, Dihydroorotase; OPRT, Orotate Phosphoribosyltransferase; UMPS, uridine monophosphate synthase; PRPP, 5-Phosphoribosyl-1-pyrophosphate; PPI, Pyrophosphate; ODC, Orotidine 5′-phosphate Decarboxylase; OMP, Orotidine 5′-monophosphate; UDP, uridine diphosphate; UTP, uridine triphosphate; dUDP, deoxyuridine diphosphate; dTTP, deoxythymidine triphos-phate; CMP, cytidine monophosphate; CDP, cytidine diphosphate; CTP, cytidine triphosphate; UCK2, uridine cytidine kinase 2; CMPK1, cytidine monophosphate kinase 1; NDPK, nucleoside diphosphate kinase; RNR, ribonucleotide reductase; CoQ_{10}, coenzyme Q_{10}; CDA, cytidine deaminase. UMP, uridine monophosphate; ORO, orotateO, ubiguinone; OI,ubiquinol; ISG, interferon-stimulated gene. NRF2, Nuclear factor erythroid 2-related factor 2; KEAP1, Kelch-like ECH-associated protein 1; NCOA4, nuclear receptor coactivator 4; GPX4, glutathione peroxidase 4; ETC, electron transport chain; CRC, colorectal cancer; AMPK, AMP-activated protein kinase; PI3K, phosphoinositide 3-kinase; JAK, Janus kinases; STAT, signal transducer and activator of transcription; ILs, interleukins; IFN, interferon; Maf, musculoaponeurotic fibrosarcoma; MDA, malondialdehyde; Trx-1, Thioredoxin 1; FMN, flavin mononucleotide; TCA, tricarboxylic acid cycle; MAT, methionine adenosyl transferase; NTP, Nucleoside triphosphates; LKB1, liver kinase B1; EC, endometrial cancer; HTA, Host-targeted antiviral drugs; MS, multiple sclerosis; T_{Naive}, naive T cells; GSH, glutathione; SAM, S-adenosyl methionine; T_{eff}, effector T cells; RA, rheumatoid arthritis; AbDHODH, *Acinetobacter baumannii* DHODH; P-AMPK, Phosphorylated Adenosine Monophosphate-activated Protein Kinase; Acetyl-CoA, Acetyl Coenzyme A; Malonyl-CoA, Malonyl Coenzyme A; ACC, Acetyl Coenzyme A carboxylase; L-Arg, L-Arginine; eNOS, Endothelial Nitric Oxide Synthase; ACSL4, Acyl-CoA Synthetase Long Chain Family Member 4; D-PUFAs, bis-allylic deuterated polyunsaturated fatty acids; PUFAs, bis-allylic polyunsaturated fatty acids; PL-OH, phospholipid hydroxide; ROS, reactive oxygen species.

Data availability

No data was used for the research described in the article.

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