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**Molecular regulatory mechanism of ferroptosis and its role in gastrointestinal oncology: Progress and updates**

Yang F *et al*. Ferroptosis in GI oncology

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**Abstract**

Gastrointestinal (GI) tumors, including liver, pancreatic, gastric, and colorectal cancers, have a high incidence rate and low survival rate due to the lack of effective therapeutic methods and frequent relapses. Surgery and postoperative chemoradiotherapy have largely reduced the fatality rates for most GI tumors, but these therapeutic approaches result in poor prognoses due to severe adverse reactions and the development of drug resistance. Recent studies have shown that ferroptosis plays an important role in the onset and progression of GI tumors. Ferroptosis is a new non-apoptotic form of cell death, which is iron-dependent, non-apoptotic cell death characterized by the accumulation of lipid reactive oxygen species (ROS). The activation of ferroptosis can lead to tumor cell death. Thus, regulating ferroptosis in tumor cells may become a new therapeutic approach for tumors, making it become a research hotspot. Current studies suggest that ferroptosis is mainly triggered by the accumulation of lipid ROS. Furthermore, several studies have indicated that ferroptosis may be a new approach for the treatment of GI tumors. Here, we review current research progress on the mechanism of ferroptosis, current inducers and inhibitors of ferroptosis, and the role of ferroptosis in GI tumors to propose new methods for the treatment of such tumors.

**Key Words:** Ferroptosis; Gastrointestinal oncology; Hepatocellular carcinoma; Pancreatic cancer; Gastric cancer; Colorectal cancer

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**Core Tip:** Ferroptosis refers to cell death triggered by iron-dependent lipid peroxidation. Recent studies have demonstrated that ferroptosis is involved in the onset and progression of numerous gastrointestinal (GI) tumors. Hence, inducing ferroptosis in tumor cells may become a new therapeutic strategy against GI tumors. Here, we review the molecular mechanism of ferroptosis and its role in GI tumors, with the aim of providing new research directions and ideas for the treatment of GI tumors.

**INTRODUCTION**

Cell death is a basic life process that is pivotal to the development and homeostasis of multicellular organisms. Functionally, cell death can be categorized into accidental cell death (ACD) and regulated cell death (RCD). ACD refers to instantaneous and catastrophic cell death due to severe physical (*e.g.*, high pressure, high temperature, and hypertonicity), chemical (*e.g.*, drastic pH fluctuations), or mechanical (*e.g.*, shear force) damage. In contrast, RCD is triggered *via* specific molecular mechanisms and can be modulated (delayed or accelerated) *via* pharmacologic or genetic interventions[1]. RCD can be further categorized by onset mechanism as apoptosis, autophagic cell death, paraptosis, mitotic catastrophe, oncosis, pyroptosis, autoschizis, necroptosis, entosis, or ferroptosis[1,2].

Ferroptosis is iron-dependent, non-apoptotic cell death characterized by the accumulation of free iron and lipid reactive oxygen species (ROS)[3]. Studies have shown that the free iron concentration in gastrointestinal (GI) tumor cells is higher than that of normal cells, and the survival of tumor cells is highly dependent on the abnormally activated antioxidant system[2,3]. Additionally, in recent years, a large number of studies have shown that the activation of ferroptosis can lead to GI tumor cell death[1-4]. Thus, regulating ferroptosis in tumor cells may become a new therapeutic approach for GI tumors. Therefore, ferroptosis has become a research hotspot.

Here, we summarize recent research progress on the mechanism of ferroptosis and its role in GI tumors to expand ideas on clinical tumor treatment.

**DISCOVERY AND CHARACTERISTICS OF FERROPTOSIS**

In 2003, Dolma *et al*[4] identified a new compound while screening for compounds with killing effects against tumor cells. The identified compound, erastin, which selectively kills tumor cells expressing RASV12 protein, a mutated form of RAS. However, the erastin-mediated killing mechanism is different from that of previously known compounds, *i.e.* it does not cause nuclear morphological changes, DNA fragmentation, or caspase-3 activation, and its cell-killing process cannot be reversed by caspase inhibitors[2]. Then Yang *et al*[5] and Yagoda *et al*[6] found that erastin-mediated cell death is inhibited by iron chelators and is accompanied by elevated intracellular ROS levels. Additionally, both studies identified RAS-selective lethal (RSL) compounds, RSL and RSL3, which trigger this type of cell death[3]. In 2012, Dixon *et al*[3] named this type of cell death ferroptosis, which is iron-dependent, non-apoptotic cell death characterized by intracellular ROS accumulation. In 2018, the Nomenclature Committee on Cell Death defined ferroptosis as a form of glutathione peroxidase 4 (GPX4)-regulated RCD that is triggered by oxidative stress in the intracellular microenvironment and can be inhibited by iron chelators and lipophilic antioxidants[1].

Ferroptosis is a novel type of iron-dependent cell death with genetic, biochemical, and morphological features different from other forms of cell death including apoptosis, unregulated necrosis, and necroptosis[3]. The ultra-micromorphological features of ferroptosis include cell membrane disruption and blebbing, mitochondrial shrinkage, increased mitochondrial bilayer density, reduced or absent mitochondrial cristae, outer mitochondrial membrane disruption, normal nuclear size, and the absence of chromatin condensation[7]. The main biochemical characteristics of ferroptosis include iron and ROS accumulation, protein kinase activation, cystine/glutamate antiporter inhibition, reduced cystine uptake and glutathione (GSH) synthesis, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidation[8].

Ferroptosis, modulated by specific pathways, is involved in various biological processes and exhibits unique gene expression and molecular regulatory systems. Current studies suggest that ferroptosis is mainly caused by the imbalance between lipid ROS generation and detoxification in cells. The accumulation of lipid ROS when the cellular antioxidant capacity is reduced can result in oxidative stress-induced cell death, *i.e.* ferroptosis[9].

**MECHANISM AND REGULATION OF FERROPTOSIS**

Ferroptosis is mainly regulated by the following three mechanisms[7]: (1) regulation of iron metabolic pathways such as autophagy-related genes 5 and 7 (ATG5/ATG7)-nuclear receptor coactivator 4 (NCOA4) pathway[10] and p62-Kelch-like epichlorohydrin-associated protein-1 (Keap1)-nuclear factor erythroid 2-related factor 2 (NRF2) pathway[11]; (2) regulation of lipid metabolic pathways such as the p53-serine acetyltransferase 1-arachidonate-15-lipoxygenase pathway[7], acyl-CoA synthase long-chain family member 4 (ACSL4)[12], lysophosphatidylcholine acyltransferase 3 (LPCAT3)[13], and 15-lipoxygenase/phosphatidylethanolamine (PE)-binding protein-1 (15-LOX/PEBP1)[14]; and (3) regulation of the GSH/GPX4 pathway such as the cystine/glutamate antiporter system (System Xc-)[15], transsulfuration pathway[16], and mevalonate pathway[17]. Dysregulation of these three regulatory pathways eventually significantly reduce GPX4 activity and increase intracellular lipid ROS levels, thereby leading to reduced cellular antioxidant capacity, additional lipid ROS accumulation, oxidative damage to the cell membrane, and ferroptosis. Ferroptosis suppressor protein 1 (FSP1) can inhibit lipid peroxidation and ferroptosis by directly eliminating lipid ROS independent of GPX4[18] (Figure 1).

***Regulation of iron metabolism***

Iron is an essential trace element in the human body. Iron deficiency can cause anemia and iron-dependent enzyme abnormalities. However, iron accumulation can lead to tissue damage and increase the risk of developing various diseases (*e.g.*, tumors). ROS that accumulate during cell metabolism mainly include superoxide radical anions (O2-) and hydrogen peroxide (H2O2), which are converted by free Fe2+ ions to hydroxyl free radicals (HO-) that subsequently generate lipid peroxides by oxidizing macromolecules, especially lipid molecules (*e.g.*, polyunsaturated fatty acids, PUFAs). These reactions, which involve iron and generate hydroxyl or alkoxy radicals (RO-), are termed Fenton reactions[19]. Intracellular accumulation of lipid peroxides without timely elimination cause oxidative damage to DNA, proteins, and the cell membrane, eventually leading to ferroptosis[20].

Therefore, iron ions are indispensable for the accumulation of lipid peroxides and the initiation of ferroptotic pathways. The absorption, distribution, metabolism, transformation, and excretion of iron ions are closely associated with the onset of ferroptosis[21]. Dietary iron is mainly absorbed as ferric (Fe3+) ions in the duodenum and upper jejunum, where it is transported to the blood by transferrin. Some Fe3+ ions are transported by binding to the membrane receptor transferrin receptor 1 (TFR1), which are packaged into endosomes. There, Fe3+ ions are reduced to Fe2+ ions by the metalloreductase, six-transmembrane epithelial antigen of the prostate 3. Finally, Fe2+ ions are delivered by solute carrier family 11a2/divalent metal transporter 1 from endosomes into the cytoplasmic labile iron pool. Intracellular iron storage mainly occurs in the form of iron-protein complexes comprising ferritin light chain (FTL) and ferritin heavy chain 1 (FTH1), while the remaining excess Fe2+ ions are oxidized to Fe3+ ions and transported out of cells by ferroportin on the cell membrane[22].

Both increased iron uptake and decreased iron elimination can enhance the sensitivity of cells to oxidative damage and ferroptosis *via* the Fenton reaction. Supplementation with exogenous iron ions but not other divalent metal ions can accelerate erastin-induced ferroptosis[3]. Cells with mutated RAS show significantly increased iron uptake and significantly decreased iron storage capacity following the onset of ferroptosis[23]. The intracellular level of labile iron (Fe2+ ions) is also a key factor affecting lipid peroxidation and ferroptosis. Upon exposure to different ferroptosis inducers, the intracellular Fe2+ ion level increases and various transport proteins associated with iron metabolism (*e.g.*, ferritin and TFR1) are rearranged after ferroptosis[21]. Iron overload and ferroptosis can be inhibited by knocking out genes encoding transferrin receptors or upregulating the expression of iron-storage proteins. Inhibiting the main transcription factor that regulates iron metabolism, iron-responsive element-binding protein 2 (also known as iron regulatory protein 2), can significantly upregulate the expression of genes associated with iron metabolism (*e.g*., FTH1 and FTL) and inhibit erastin-induced ferroptosis[24]. Blocking iron transport by knocking out the ferroportin gene SLC11A3 exacerbates erastin-induced ferroptosis in neuroblastoma cells[25]. Furthermore, Yang *et al*[26] observed that phosphorylase kinase catalytic subunit γ2 (PHKG2) positively regulates ferroptosis by modulating the free Fe2+ ion level, while inhibiting PHKG2 expression exhibits an iron-chelating effect. Autophagy can also regulate the cellular sensitivity to ferroptosis by affecting iron metabolism[27]. Ferritin-selective autophagy (ferritinophagy) enhances cellular sensitivity to ferroptosis by controlling the level of available iron[28]. NCOA4 is a selective cargo receptor that delivers ferritin to autophagosomes, where ferritin is degraded and free iron is released into the cytoplasm. Downregulating NCOA4 expression reduces the sensitivity of human fibrosarcoma cells (HT-1080) and human pancreatic cancer cells (PANC1) to ferroptosis. This process is regulated by autophagy-related genes, ATG5 and ATG7[10]. Other proteins that affect iron metabolism, such as NRF2[11], heat shock protein beta-1[29], and CDGSH iron-sulfur domain-containing protein 1 (CISD1, also referred to as mitoNEET)[30] can also affect cellular sensitivity to ferroptosis.

Iron chelators can directly act on iron-containing enzymes, most likely lipoxygenases, because they can catalyze PUFA oxidation and be directly inactivated by lipophilic iron chelators. Dixon *et al*[31] suggested that iron is extremely prone to electron exchange under aerobic conditions. Thus, the inhibition of ferroptosis by iron chelators may be attributed to the fact that iron is a cofactor of numerous important metalloenzymes and that iron chelators prevent electron transfer from iron to oxides, thereby inhibiting oxygen free radical generation and preventing ferroptosis by inhibiting lipid peroxidation. Therefore, regulating iron metabolism and ferritinophagy may serve as a new target and approach for modulating ferroptosis.

***Regulation of lipid metabolism***

Lipids are important regulators of cell death. In mammals, both apoptotic and non-apoptotic pathways can be induced, regulated, or inhibited by different lipid signals[34]. For example, increasing the intracellular saturated fatty acid-to-monounsaturated fatty acid ratio can trigger apoptotic pathways. Increased long-chain fatty acid levels can trigger necrotic pathways[32], and exogenous monosaturated fatty acids can reduce cell death *via* acyl-CoA synthetase long chain family member 3 (ACSL3). All of these pathways exert a lipotoxic effect[33].

Unlike other forms of cell death, ferroptosis does not require an effector (*e.g.*, pore-forming proteins). Instead, lipid-mediated oxidative stress and subsequent membrane damage are key factors leading to the onset of ferroptosis. In particular, PUFAs, which contain bis-allylic protons that are vulnerable to hydrogen abstraction, are more likely to form lipid peroxides and induce ferroptosis[26]. PUFA abundance and localization determine the degree of lipid peroxidation in cells, and thus, the extent of ferroptosis[7].

The intracellular accumulation of lipid peroxides is the core process of ferroptosis. Lipid peroxidation in cells may be enzymatic or non-enzymatic. Non-enzymatic lipid peroxidation, also known as lipid autoxidation, is a free radical-mediated chain reaction, in which PUFAs are oxidized to lipid hydroperoxides by hydroxyl radicals generated *via* the Fenton reaction[19]. In contrast, enzymatic lipid peroxidation refers to lipoxygenase (LOX)-catalyzed generation of various lipid hydroperoxides from free PUFAs. Then lipid hydroperoxides are catalyzed by Fe2+ ions to generate free alkoxy radicals, which participate in the next lipid peroxidation reaction. Continuous PUFA oxidation and depletion alters the fluid mosaic structure of cell membrane and increases its permeability, eventually leading to cell death[17,20].

Fe2+ ions participate in the formation of free radicals and are an important catalyst in lipid peroxidation. Free PUFAs serve as substrates for the synthesis of lipid signaling mediators, but they must be esterified to membrane phospholipids and oxidized to become ferroptotic signals[17]. These toxic mediators are sparsely distributed within the cell membrane, mitochondrial membranes, lysosomal membranes, and endoplasmic reticulum membranes[26]. A lipidomic study uncovered that lipid metabolism disorders are closely associated with ferroptosis, where the key phospholipids—PEs—which contain arachidonic acid (C20:4) or its derivative adrenic acid (C22:4), are oxidized to ox-phosphatidylethanolamines (ox-PEs) that induce the onset of ferroptosis[34]. PUFAs are converted to coenzyme A derivatives, which are incorporated into phospholipids to become ferroptotic signals. Thus, the regulatory enzymes involved in PUFA biosynthesis from membrane phospholipids can trigger or prevent ferroptosis. Indeed, PUFA formation requires various lipid metabolism enzymes, such as ACSL4[14], LPCAT3[13], and 15-LOX/PEBP1[14]. In addition, lipid peroxidation promotes ferroptosis due to the generation of toxic aldehydes, such as 4-hydroxy-2-nonenal and malondialdehyde that can inactivate some proteins involved in normal physiological functions[35].

PUFAs are activated by ACSL4 and transported by LPCAT3 to the inner and outer leaflets of the cell membrane, where they undergo esterification and participate in the oxidation of negatively charged membrane phospholipids. Under normal circumstances, 15-LOX/PEBP1 and GPX4 co-regulate the oxidation of esterified fatty acids, but during oxidant/antioxidant imbalance, long-chain PUFAs in the cell membrane are often oxidized and trigger ferroptosis, especially when being induced by other factors such as RSL3[22].

ACSL4, which belongs to the long-chain acyl-CoA synthetase family, catalyzes the activation of fatty acids to form fatty acyl CoA in the body. It is also the key enzyme required in the first step of fatty acid catabolism. Previous studies have revealed that knocking out enzymes of the ACSL family other than ACSL4 in mouse embryonic fibroblasts does not cause ferroptosis[12]. Unlike other members of the ACSL family, ACSL4 can activate long-chain PUFAs and participate in the synthesis of membrane phospholipids. For example, ACSL4 catalyzes the conversion of arachidonic acid and adrenergic acid to arachidonoyl-CoA and adrenyl-CoA, respectively, which participate in the synthesis of negatively charged membrane phospholipids (*e.g.*, phosphatidylethanolamines and phosphatidylinositol) and their incorporation into the cell membrane. LPCAT3 knockout cells display only a slight alleviation of ferroptosis compared to ACSL4 knockout cells. Additionally, ACSL4 is required for lipid peroxides to inhibit GPX4[12,36]. These results suggest that ACSL4 may be a crucial determinant of ferroptosis. Another study revealed that thiazolidinediones exhibit a protective effect on ACSL4-knockout embryonic fibroblasts. The combination of thiazolidinediones and RSL3 alleviated membrane lipid oxidation and cell death and significantly improved the survival of ACSL4-knockout mice[12]. Hence, ACSL4 inhibition may be a new target for the treatment of diseases associated with ferroptosis.

LOXs are non-heme, iron-containing enzymatic effector proteins essential for mediating the formation of ferroptosis-related peroxides. Knocking out LOXs, which prefer free PUFAs as substrates, can alleviate erastin-induced ferroptosis and cellular damage[3]. Vitamin E can inhibit LOX activity, which provides a foundation for the protective effect of vitamin E against ferroptosis[37]. Current studies suggest that LOXs primarily form a complex with PEBP1, which allosterically regulate LOXs to accommodate the ferroptotic signal sn2-15-Hydroperoxy-eicasotetraenoyl-phosphatidylethanolamines (sn2-15-HpETE-PE) at the catalytic site. Two major LOX subtypes mediate lipid peroxidation: 15-LO1 and 15-LO2. These two LOX subtypes have tissue-specific distribution patterns. For example, 15-LO1 is highly expressed in human aortic endothelial cells, while 15-LO2 is highly expressed in renal tubular endothelial cells and neuronal cells[14]. A previous redox metabolomic analysis revealed the similarity between 15-LO1 and 15-LO2. Both enzymes are involved in ferroptosis-associated diseases, such as traumatic brain injury, asthma, and acute renal ischemic injury[7]. LOX-mediated free PUFA oxidation requires 15-LOX/PEBP1 complex formation. In this complex, PEBP1 allosterically regulates LOXs and initiates downstream phospholipase A2-related oxidation pathways for specific PUFAs. PEBP1, also known as RAF1 kinase inhibitor protein, is a small scaffold protein that binds to RAF1 and inhibits activity under steady-state conditions. 15-LOs are newly identified partners of PEBP1. 15-LO/PEBP1 complexes allosterically activate LOXs, which convert 15-hydroxyperoxyeicosatetraenoic acid (15-HpETE) to the pro-ferroptotic signal, 15-HpETE-PE, thereby triggering ferroptosis[38]. The mechanism by which the LOX/PEBP1 complex selects specific PUFAs for oxidation among diverse unsaturated fatty acids remains unknown. Clearly, this issue urgently needs to be addressed in investigating the regulatory mechanism of ferroptosis.

***Regulation of amino acid metabolism***

Amino acid metabolism is an important component of metabolic networks, and amino acid metabolism disorders are closely associated with ferroptosis[7]. GSH is an important antioxidant and free radical scavenger in the body. Many free radicals produced *via* metabolism can damage cell membranes, attack biological macromolecules, promote aging, and induce the onset of tumors or atherosclerosis. Functionally, GSH can bind and convert harmful, toxic molecules (*e.g.*, free radicals and heavy metals) into harmless substances that can be excreted from the body[39]. GSH is a tripeptide consisting of three amino acid residues: glutamic acid, cysteine, and glycine. It exists in reduced (G-SH) and oxidized (G-S-S-G) forms and is the first line of defense for free-radical scavenging in the body due to the presence of an active sulfhydryl (-SH) group that is susceptible to oxidization and dehydrogenation. Together with non-enzymatic antioxidants (reduced nicotinamide adenine dinucleotide phosphate/nicotinamide adenine dinucleoside phosphate), GSH exerts a strong protective effect on the body[40]. The synthesis of GSH requires cysteine as the starting material. Therefore, cellular resistance to lipid oxidation relies on intracellular cysteine levels, which are mainly produced by the System Xc- and transsulfuration pathways.

System Xc- plays an important role in maintaining GSH homeostasis and distribution. This molecule is a disulfide-linked heterodimer that comprises the regulatory subunit solute carrier family 3 member 2 (SLC3A2) and the catalytic subunit solute carrier family 7 member 11 (SLC7A11). System Xc- promotes a 1:1 cystine and glutamic acid exchange across the plasma membrane. Cystine is reduced to cysteine upon entering cells[41]. Thus, System Xc- regulates GSH synthesis by affecting extracellular glutamic acid levels[42]. A previous study found that System Xc--knockout mice have significantly lower glutamic acid levels around neurons and a milder drug-induced neurotoxic response than normal mice[43]. Previous pharmacological studies revealed that erastin, sulfasalazine, and high glutamic acid concentrations induce ferroptosis by inhibiting System Xc-[3,44]. These findings indicate that System Xc- may mediate ferroptosis initiation by affecting glutamic acid uptake and GSH synthesis.

Methionine can be converted to adenosylhomocysteine and cysteine in cells *via* the transsulfuration pathway[16]. During cysteine insufficiency, homocysteine is converted to cystathionine (a cysteine precursor), which eventually enters the cysteine pool *via* the transsulfuration pathway. Numerous studies have demonstrated that more than 40% of cysteine in mammals is obtained from food. Cysteine is mainly used to synthesize GSH, antioxidant peptides, and thioredoxin (Trx) in the body. Under oxidative stress, cystathionine-b-synthetase promotes the conversion of methionine to cysteine and subsequent GSH synthesis, thereby protecting cells from oxidative stress-induced damage[16,45]. Hence, cysteine can be synthesized in cells *via* the transsulfuration pathway even when intracellular System Xc- is inhibited, indicating that ferroptosis inducers, which inhibit System Xc-, cannot completely and effectively kill cells. Hayano *et al*[46] showed that inhibiting cysteinyl-tRNA synthetase (CARS) expression using RNA interference upregulates the transsulfuration pathway and enhances cellular resistance to erastin-induced ferroptosis but is unable to inhibit RSL3- or buthionine sulfoximine-induced ferroptosis, suggesting that the transsulfuration pathway negatively regulates ferroptosis.

Glutamic acid and glutamine are additional ferroptosis regulators. A high extracellular concentration of glutamic acid can inhibit System Xc- and trigger ferroptosis. Ottestad-Hansen *et al*[43] found that knocking out System Xc- protects mice against neurotoxic injuries caused by glutamic acid accumulation. Additionally, iron chelators and ferroptosis inhibitors can inhibit glutamic acid-mediated neurotoxicity. Glutamine naturally exists in human tissues and plasma at substantial concentrations. Its degradation fuels the tricarboxylic acid cycle and provides fundamental materials for biosynthetic processes. During glutamine deficiency or the inhibition of glutamine degradation, ROS accumulation, lipid peroxidation, and ferroptosis cannot be induced by depleting cysteine or blocking cystine uptake, probably because the product of glutamine degradation, α-ketoglutarate, is essential for the onset of ferroptosis[3]. However, not all glutamic acid metabolic pathways can induce ferroptosis. The first step of glutamic acid metabolism is the conversion of glutamine into glutamic acid by the glutaminases GLS1 and GLS2. These glutaminases have similar structures and enzymatic properties, but only GLS2 can induce ferroptosis, probably because GLS2 is a transcriptional target of p53. Indeed, GLS2 upregulation can induce p53-dependent ferroptosis[47,48]. Under certain circumstances, p53 can suppress ferroptosis by blocking dipeptidyl-peptidase-4 activity in a transcription-independent manner[49]. Inhibiting glutamine degradation has been demonstrated to alleviate cardiac, renal, and brain injury caused by ischemia-reperfusion in an experimental model[50]. Hence, the regulation of glutamine anabolism may provide new approaches for alleviating ferroptosis-induced organ injuries.

In addition, oxidant/antioxidant imbalance may also induce ferroptosis[7]. ROS levels in the body are regulated by an antioxidative defense system comprising antioxidants, such as Nrf2, GPX4, and catalase. However, inhibitors of the antioxidative system (*e.g.*, superoxide dismutase inhibitors and thioredoxin reductase inhibitors) can induce human epithelial/fibroblast cell death only when intracellular GSH is depleted[51], indicating that erastin may induce ferroptosis by interacting with a specific downstream target of GSH. GPX4, which belongs to the GPX antioxidative defense system, is a key enzyme in maintaining the balance between GSH and GS-SG. High SLC7A11 expression in various tumor types increases cystine uptake and GPX4 synthesis in cells, thereby promoting tumor growth by reducing cellular oxidative stress and inhibiting ferroptosis[41].

GPX4 is a GSH-dependent enzyme. Selenocysteine is an amino acid within the catalytic center of GPX4, but since it is encoded by a UGA codon (which is also a stop codon), selenocysteine needs to be inserted into GPX4 by a specific carrier. Selenocysteine-specific tRNA (sec-tRNA) contains isopentenyladenosine and can decode the selenocysteine UGA codon, thereby allowing the accurate insertion of selenocysteine into corresponding proteins. Importantly, sec-tRNAmaturation can also be regulated by the mevalonate pathway acting on GPX4 because its maturation requires tRNA-isopentenyltransferase to catalyze the transfer of the isopentenyl group of isopentenyl pyrophosphate (IPP) to the specific adenine sites of sec-tRNA precursors. Since IPP is an important product of the mevalonate pathway, inhibitors of the mevalonate pathway (*e.g.*, statins) can inhibit sec-tRNA maturation and GPX4 synthesis[16,17], thereby affecting the progression of ferroptosis. IPP and mevalonate pathway inhibitors regulate the onset of ferroptosis by affecting GPX4. At present, GPX4 is a key target to induce ferroptosis and is activated by numerous ferroptosis inducers, such as erastin and RSL3. Erastin inhibits GPX4 activity by depleting GSH, while RSL3 directly inhibits GPX4 activity[7], resulting in lipid peroxide accumulation that triggers ferroptosis. Additionally, other ferroptosis inducers (*e.g.*, diphenylene iodonium (DPI), DPI7, DPI10, and DPI12) exert similar effects by directly inhibiting GPX4 activity. Knocking out GPX4 Leads to excess intracellular lipid peroxide accumulation and cell death[52]. Therefore, GPX4 is an important target for triggering ferroptosis.

**OTHER REGULATORY PATHWAYS**

***FSP1/CoQ/NADPH pathway***

In addition to the above-mentioned metabolic regulatory pathways, other cellular pathways are involved in the regulation of ferroptosis. Bersuker *et al*[18] and Doll *et al*[53] found that the FSP1/coenzyme Q (CoQ)/NADPH pathway also inhibits ferroptosis. FSP1 was previously known as apoptosis-inducing-factor mitochondria-as-2. Both research groups found that FSP1 exhibited CoQ oxidoreductase activity, which mediates NAD(P)H-dependent CoQ10 regeneration. Ubiquinol, the reduced form of CoQ10, captures free radicals that drive lipid peroxidation, thereby preventing oxidative damage to plasma membranes. FSP1 exerts its cellular protective effect against ferroptosis by catalyzing continuous CoQ10 regeneration and improving the free-radical scavenging capacity within cells. Hence, FSP1 catalyzes the synthesis of lipophilic free-radical scavengers and has a protective effect against ferroptosis caused by GPX4 deletion. It is currently believed that the FSP1/NADPH/CoQ10 pathway is independent and parallel to GPX4. Even in the absence of GPX4, FSP1, CoQ10, NADPH, and GSH serve as important antioxidants and free-radical scavengers that exhibit cellular protective effects against ferroptosis in the body.

***Voltage-dependent anion channels***

Voltage-dependent anion channels (VDACs) are transmembrane channels located on the outer mitochondrial membrane that transport ions and metabolites. VDACs regulate mitochondrial metabolism and energy production and participate in regulating signaling pathways, leading to both cell survival and death. There are numerous VDAC subtypes including VDAC1, VDAC2, and VDAC3. The open state of VDACs mediates the influx of respiratory substrates, ADP, and phosphoric acid into the mitochondria, while its closure blocks transport across mitochondrial membranes[54]. Tubulin, a globular protein on VDACs, can dynamically regulate mitochondrial metabolism and ion transportation by blocking VDACs[55]. Tubulin-induced VDAC closure restricts metabolite influx into the mitochondria and limits ATP production, leading to attenuated oxidative stress due to the inhibition of mitochondrial metabolism and a relatively low ATP/ADP ratio. Erastin can inhibit the effect of tubulin on VDACs and maintain an open state by preventing free tubulins in the cytoplasm from blocking VDACs. The open VDAC state leads to increased mitochondrial metabolism, decreased glycolysis, and elevated ROS production. Exposure of VDACs to the ferroptosis inducer, erastin, causes increased permeability of outer mitochondrial membranes, membrane ion channel opening, and disrupted cellular homeostasis, which results in dysfunctional mitochondrial metabolism and oxidation, increased ROS production, and enhanced lipid peroxidation, eventually triggering ferroptosis[56]. A previous study showed that inhibiting VDAC2 or VDAC3 expression renders cells insensitive to erastin-induced ferroptosis, but upregulating VDAC2 or VDAC3 expression does not significantly increase cellular sensitivity to erastin-induced ferroptosis. These data suggest that despite being involved in the regulation of ferroptosis, neither VDAC2 nor VDAC3 is a prerequisite of ferroptosis[57]. VDAC1 is closely related to the onset of ferroptosis, as it mainly maintains calcium homeostasis and ROS levels in the mitochondria.

**FERROPTOSIS INDUCERS AND INHIBITORS**

***Common ferroptosis inducers***

Ferroptosis inducers can be divided into four categories according to their targets (Table 1): System Xc-; GPX4; GSH; and iron ions and ROS.

***Common ferroptosis inhibitors***

Ferroptosis inhibitors can be divided into two categories according to their mechanisms of action (Table 2): reduction of intracellular iron accumulation; and inhibition of lipid peroxidation.

**FERROPTOSIS AND GI DISEASES**

Numerous studies have demonstrated that ferroptosis leads to cell death in GI tumors (*e.g.*, pancreatic, liver, colorectal, and gastric cancers) and plays an important role in inhibiting tumor growth. Therefore, inducing ferroptosis in tumor cells is expected to become a novel therapeutic strategy. Although only limited *in vitro* and *in vivo* experiments on ferroptosis inducers have been conducted, a few small-molecule ferroptosis inducers have been discovered that display excellent therapeutic or synergistic outcomes against tumors.

***Ferroptosis and pancreatic cancer***

Pancreatic cancer is a highly malignant GI tumor with a poor prognosis. Although there are drugs available to treat pancreatic cancer, patients receiving pharmacotherapy rarely survive more than 6 mo. Gemcitabine is the first-line chemotherapeutic agent for pancreatic cancer, but pharmacotherapy and immunotherapy still fail to yield an ideal therapeutic outcome. Therefore, it is imperative to develop new strategies for enhancing the sensitivity of pancreatic cancer to immunotherapy and reducing its resistance to gemcitabine[58]. Tang *et al*[59] utilized public databases to systematically analyze the expression of 43 ferroptosis regulators in 31 cancer types and constructed a highly accurate prognostic prediction model for pancreatic cancer based on ferroptosis regulators. A follow-up investigation on the effect of ferroptosis on the tumor microenvironment revealed that tumors that are highly sensitive to ferroptosis may also be sensitive to immune checkpoint inhibitors and *vice versa*. The authors also found that gemcitabine-resistant cancer cells had increased expression levels of SLC7A11 and SLC3A2, but their effects on ferroptosis sensitivity require further investigation. Zhu *et al*[60] found that heat shock protein family A55 (HSPA5) is closely associated with the prognosis of pancreatic cancer patients who received gemcitabine treatment. Activating the HSPA5-GPX4 pathway in pancreatic cancer cells may lead to gemcitabine resistance that may be reversed by inhibiting HSPA5 or GPX4 expression, which may also induce ferroptosis. Shintoku *et al*[61] demonstrated that erastin and RSL3 can induce pancreatic cancer cell death, and LOXs can increase the sensitivity of tumor cells with mutated RAS to erastin and RSL3. A subsequent study by Kuang *et al*[62] showed that the redox regulator quinazolindione (QD) inhibits pancreatic cancer cell proliferation by inducing ferroptosis. Further, the compound QD325 significantly inhibits the growth of transplanted tumors in mice and is well tolerated *in vivo*. Kasukabe *et al*[63] showed that the combination of cotylenin A (CN-A) and phenethyl isothiocyanate significantly inhibits pancreatic cancer cell proliferation by promoting ferroptosis. A study carried out by Yamaguchi *et al*[64] suggested that piperlongumine could synergistically kill human pancreatic cancer cells with CN-A or sulfasalazine *via* ferroptosis. In recent years, some Chinese herbal medicines have also been found to exert antitumor effects by inducing ferroptosis. Previous *in vitro* and *in vivo* assays showed that the antimalarial drug, artesunate, could cause excessive intracellular ROS accumulation by promoting lipid peroxidation and regulating iron metabolism. Additionally, artesunate can specifically induce ferroptosis in pancreatic cancer cells with a mutated *Kras* gene while exerting minimal toxic effects on normal cells[65], primarily by increasing ROS production[51]. A further study revealed that inhibiting glucose regulatory protein 78 expression reverses the resistance of pancreatic cancer cells to ferroptosis and enhances the sensitivity of tumors to artesunate[66]. The animal model constructed by Badgley *et al*[67] showed that therapeutic cysteine depletion can induce ferroptosis in pancreatic tumors in mice with mutated *Kras*/*p53*. However, Dai *et al*[68] recently found that ferroptosis can promote dead cancer cells to release KRAS protein, which will then be packaged into exosomes and taken up by macrophages. Then, the macrophages undergo polarization to M2 macrophages, which promote the malignant growth of pancreatic cancer. These results indicate that ferroptosis may exhibit complicated biological effects in the treatment of pancreatic cancer.

***Ferroptosis and liver cancer***

Surgery is the most important therapeutic approach for patients with hepatocellular carcinoma (HCC), but the rate of postoperative recurrence and metastasis is relatively high. Sorafenib is a commonly used chemotherapeutic drug against HCC, but it is difficult to clinically determine the prognosis of HCC and reduce sorafenib resistance[69]. Shan *et al*[70] analyzed two different public HCC databases and found that ubiquitin-like modifier activating enzyme 1 (UBA1) can regulate ferroptosis in HCC cells *via* the Nrf2 pathway. The authors subsequently confirmed that silencing UBA1 gene expression inhibits HCC proliferation, migration, and invasion, increasing Fe2+ and MDA levels in cancer cells. These results indicate that UBA1 can be used as an independent indicator of liver cancer progression. Liang *et al*[71] systematically analyzed the expression of 60 ferroptosis-associated genes in HCC tumor tissues and their relationships with the overall survival of patients. The authors proposed and validated a prognostic model comprising 10 ferroptosis-associated genes (ACACA, ACSL3, CISD1, CARS, G6PD, GPX4, NQO1, NFS1, SLC7A11, and SLC1A5). These efforts provided an important approach for elucidating mechanisms underlying HCC development and predicting its prognosis.

Studies on the mechanisms through which sorafenib and erastin induce ferroptosis in HCC have provided new approaches for addressing chemotherapeutic drug resistance. Louandre *et al*[72] showed that sorafenib-treated HCC cells had significantly lower retinoblastoma protein expression than untreated HCC cells, with a mortality rate two to three times higher than that of the untreated group. Subsequently, *in vivo* experiments on mice implanted with HCC cells and *in vitro* experiments on shRb-transfected Huh7 cells clarified the mechanism through which Rb regulates sorafenib-induced ferroptosis in HCC. Sorafenib induces ferroptosis in HCC by enhancing mitochondrial ROS generation, while Rb inactivation aggravates ferroptosis by increasing mitochondrial ROS levels and oxidative stress. Sun *et al*[11] reported that the p62-Keap1-Nrf2 signaling pathway plays an important role in erastin/sorafenib-induced ferroptosis in HCC, where it modulates ferroptosis by regulating the expression of downstream iron- and ROS metabolism-related genes. Interfering with p62 expression can enhance erastin/sorafenib-induced ferroptosis in HCC. Additionally, experiments in Nrf2-shRNA-transfected HCC cells and mice implanted with Nrf2-shRNA-transfected HCC cells showed that Nrf2 knockdown enhances the antitumor activity of erastin/sorafenib against HCC[73]. Qi *et al*[74] found that erastin significantly inhibits the expression of GA binding protein transcription factor subunit β 1 (GABPB1) protein and peroxidase genes in HCC cells, thereby resulting in intracellular ROS and malondialdehyde accumulation, which leads to cell death. Therefore, GABPB1 may be a key molecule that mediates erastin-induced ferroptosis in HCC. Further, ACSL3 and ACSL4 expression is significantly upregulated in HCC[75], and ACSL4 contributes to erastin-induced ferroptosis *via* 5-hydroxyeicosatetraenoic acid-mediated lipotoxicity[14]. Additional studies[76-78] have shown that inhibiting metallothionein 1G and oxidative stress-related protein sigma 1 receptor enhances the sensitivity of liver cancer cells to sorafenib by inducing ferroptosis. Wang *et al*[79] identified and explored branched-chain amino acid aminotransferase 2 (BCAT2), which is involved in System Xc- inhibitor-induced ferroptosis in liver cancer. In addition, BCAT2 also participates in ferroptosis synergistically induced by sulfasalazine and sorafenib.

Combination therapy may improve the clinical outcomes of patients with liver cancer by partially addressing the issue of drug resistance. Low-density lipoprotein nanoparticles reconstituted with the natural omega-3 PUFA, docosahexaenoic acid (LDL-DHA), can effectively kill liver cancer cells by triggering ferroptosis[80]. The combined treatment of liver cancer cells with erastin, sorafenib, and haloperidol can elevate intracellular iron ion concentrations, which generate excessive ROS *via* the Fenton reaction and increase lipid oxidation, thereby inducing ferroptosis in liver cancer cells[77]. Shang *et al*[81] found that ceruloplasmin (CP) inhibits ferroptosis by regulating iron homeostasis in HCC cells, while inhibiting CP significantly increases intracellular Fe2+ and ROS accumulation, thereby promoting erastin- and RSL3-induced ferroptosis in HCC. Li *et al*[82] reported that sorafenib and artesunate synergistically suppress liver cancer by inducing ferroptosis. Further, nanoparticle-based drugs also offer a new direction for *in situ* induction of ferroptosis in liver cancer. Tang *et al*[83] showed that manganese-silica nanodrugs induce ferroptosis in tumor cells by rapidly depleting intracellular GSH. LDL-DHA nanoparticles increase lipid peroxidation in liver cancer cells, reduce GSH levels, and inhibit GPX4 activity, thereby inducing ferroptosis that kills liver cancer cells and inhibits the *in situ* growth of liver tumors in rats[80].

***Ferroptosis and gastric cancer***

Gastric cancer (GC) is among the most common causes of cancer-related deaths worldwide, with nearly one million cases diagnosed each year and more than 730000 deaths. Conventional treatments for GC include surgery, chemotherapy, and radiotherapy. Chemotherapy, despite being the primary therapeutic approach, causes significant side effects for most patients and often cannot cure patients with advanced GC[84]. Therefore, it is necessary to develop a better therapeutic approach for GC. Lee *et al*[85] found that the sensitivity of GC cells to ferroptosis depends on PUFA biosynthesis. Stearoyl-CoA desaturase 1 (SCD1) promotes tumor growth and makes GC cells resistant to ferroptosis. Notably, GC patients with high SCD1 expression may not have an optimistic prognosis. Taken together, this study provides new insights into the potential of SCD1 as a biomarker and therapeutic target for GC[86]. Hao *et al*[87] found that inhibiting cysteine dioxygenase 1 (CDO1) expression could inhibit ferroptosis in GC by upregulating GPX4 expression and preventing ROS production. Sun *et al*[88] showed that perilipin2 inhibits ferroptosis in GC by regulating ACSL3 and 15-LOX. Some ingredients of Chinese medicines, such as *Actinidia chinensis* planch[89] and Tanshinone IIA[90] also exhibit anticancer effects against GC by participating in ferroptosis.

***Ferroptosis and colorectal cancer***

Colorectal cancer (CRC) is the most common malignant GI tumor that poses a major threat to human health. Recently, an increasing trend in CRC incidence and fatality rates has been observed, resulting from improved living standards and dietary changes[91,92]. A previous study showed that the ferroptosis inducer RSL3 triggers ferroptosis in various CRC cell types by affecting GPX4 activity in a dose- and time-dependent manner[93]. Acyl-CoA dehydrogenase, short/branched chain (ACADSB), which belongs to the acyl-CoA dehydrogenase family, reduces GSH concentration by negatively regulating GSH reductase and GPX4 expression. Further, ACADSB affects CRC cell migration, invasion, and proliferation by regulating ferroptosis[94]. Another study on ferroptosis-related mechanisms in CRC laid the foundation for the development of anticancer drugs against CRC. Park *et al*[95] showed that bromelain affects ferroptosis by regulating ACSL4 expression in CRC cells with Kras mutations. Additionally, talaroconvolutin A[96], 2-imino-6-methoxy-2H-chromene-3-carbothioamide[97], and resibufogenin[98] have been found to inhibit CRC cell proliferation and tumorigenesis by modulating ferroptosis in CRC cells. Some studies also found that combination therapy could partially address the issue of CRC resistance to chemotherapeutic drugs *via* ferroptosis. Andrographis enhances the sensitivity of CRC cells to 5-fluorouracil by promoting ferroptosis[99]. The combination therapy using the natural products β-elemene and cetuximab can kill CRC cells with mutated KRAS genes by inducing ferroptosis and inhibiting epithelial-mesenchymal transition[100]. The combination of high-dose vitamin C and cetuximab can improve the drug sensitivity of CRC by triggering ferroptosis, thereby laying the foundation for the treatment of CRC[101].

**CONCLUSION**

Ferroptosis has received increasing attention since being proposed as a form of RCD by Dixon*et al*[3] in 2012. Numerous in-depth studies have been conducted on the complex molecular mechanisms underlying ferroptosis. These studies facilitate a deeper understanding of the onset and progression of ferroptosis-associated diseases. The further development of relevant targeted drugs has also led to the emergence of a new research field associated with ferroptosis onset and progression for the treatment of GI tumors[59]. Following the discovery of erastin in 2003, numerous ferroptosis inducers and inhibitors have been identified because of the increasing importance of the relationship between ferroptosis and GI tumors[7]. Sorafenib, the sole first-line drug for liver cancer, is believed to kill hepatocytes *via* ferroptosis. Additionally, some *in vitro* and *in vivo* drug trials on pancreatic cancer have provided new theoretical bases and research directions for the pharmacotherapy of pancreatic cancer. Some studies on ferroptosis in GC and CRC indicated that inducing ferroptosis could cause cell death in GI tumors and exert a synergistic effect with other anticancer drugs, thereby enhancing tumor sensitivity to existing treatments. Hence, inducing ferroptosis may have considerable potential for treating GI tumors[102]. However, research on ferroptosis is still at a preliminary stage, and it is of great theoretical and practical significance to continuously explore the mechanisms and roles of ferroptosis in various diseases. These studies will reveal highly effective and targeted therapeutic approaches. For instance, the mechanism and key regulators of ferroptosis as well as its relationships with tumor-associated genes and other RCDs (*e.g.*, autophagy and apoptosis), are potential directions and goals for future studies. Collectively, these studies will facilitate an in-depth understanding of the molecular mechanism through which GI tumors evade cell death and promote the development of novel effective therapeutic strategies. Therefore, further discoveries and investigation of ferroptosis inducers and inhibitors will provide a theoretical foundation and new method for the treatment of GI tumors.

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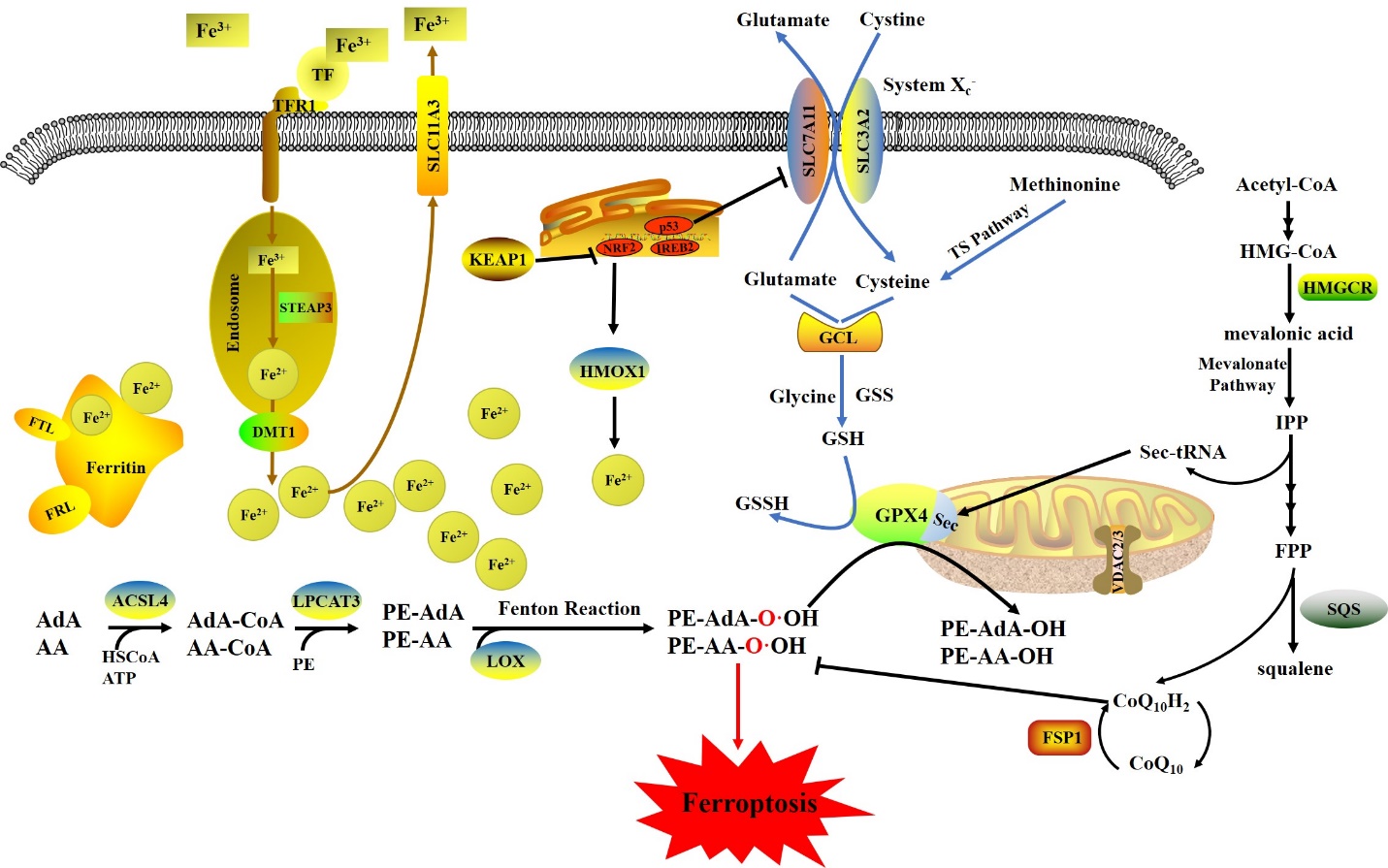
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**Figure Legends**



**Figure 1 Molecular regulation mechanism of ferroptosis.** ART: Artesunate; DHA: Dihydroartemisinin; GPX4: Glutathione peroxidase 4; GSH: Glutathione; HMOX1: Heme oxygenase 1; PUFAs: Polyunsaturated fatty acids; ROS: Reactive oxygen species; SQS: Squalene synthase; VDAC: Voltage-dependent anion channel.

**Table 1 Common ferroptosis inducers**

|  |  |  |  |
| --- | --- | --- | --- |
| **Target** | **Inducer** | **Function** | **Ref.** |
| System Xc- | Erastin | Inhibits the activity of System Xc- and affects the synthesis of GSH; binds to VDAC2/3 to induce mitochondrial dysfunction | [3,6] |
| Erastin analogs, piperazine erastin, imidazole ketone erastin | Inhibits the activity of System Xc- and affects the synthesis of GSH | [103,104] |
| Sulfasalazine | Inhibits the activity of System Xc- (weaker inhibitory effect than erastin) | [3,105,106] |
| Sorafenib | Inhibits the activity of System Xc- (directly affects the synthesis of GSH in a narrow concentration range) | [107] |
| Glutamate | Inhibits the activity of System Xc-, high extracellular glutamate concentrations prevent cystine import | [3,7] |
| GPX4 | (1S,3R)-RSL3 | Covalently binds to the selenocysteine residue of GPX4 | [5,26] |
| DPI7 (ML162), DPI12, DPI17 | Covalently bind to GPX4 (at the same binding site as RSL3) | [7,26] |
| DPI10 (ML120), DPI13 | Indirectly inhibit GPX4 activity or bind to a site different from RSL3 | [26,103] |
| FIN56 | Induces GPX4 degradation; binds and activates SQS to deplete CoQ10 | [108] |
| Altretamine | Inhibits the activity of GPX4 | [64] |
| GSH | BSO | GSH depletion | [7,103] |
| Cisplatin | Binds to GSH to inactivate GXP4 | [109] |
| DPI2 | Depletes GSH | [7,103] |
| Cysteinase | Depletes cysteine, resulting in GSH depletion | [7] |
| Piperlongumine | Depletes GSH and inhibits the activity of GXP4 | [7,64] |
| ROS and iron | FINO2 | Oxidizes Fe2+ ions and promotes intracellular accumulation of ROS; indirectly inactivates GPX4; directly oxidizes PUFAs | [110] |
| Ferric ammonium citrate | Increases iron abundance | [7] |
| Silica-based nanoparticles | Delivers iron into cells and reduce GSH abundance | [7] |
| Heme | Upregulates HMOX1 expression and increases the intracellular level of labile iron | [111] |
| ART, DHA | Oxidize Fe2+ ions and promote intracellular accumulation of ROS; induce ferritinophagy and the release of labile iron | [7,51,65] |
| Siramesine and lapatinib | Downregulate FPN expression and upregulate TRF expression to increase intracellular labile iron levels | [112] |

ART: Artesunate; DHA: Dihydroartemisinin; GPX4: Glutathione peroxidase 4; GSH: Glutathione; HMOX1: Heme oxygenase 1; PUFAs: Polyunsaturated fatty acids; ROS: Reactive oxygen species; SQS: Squalene synthase; VDAC: Voltage-dependent anion channel.

**Table 2 Common ferroptosis inhibitors**

|  |  |  |  |
| --- | --- | --- | --- |
| **Target** | **Inhibitors** | **Function** | **Ref.** |
| Lipid peroxidation | Vitamin E, α-tocopherol, trolox, tocotrienols | Block propagation of lipid peroxidation, may inhibit lipoxygenases | [7] |
| Deuterated polyunsaturated fatty acids | Block initiation and propagation of lipid peroxidation | [7] |
| Butylated hydroxytoluene, butylated hydroxyanisole | Block lipid peroxidation | [7] |
| Ferrostatins, liproxstatins | Scavenge ROS and inhibit lipid peroxidation; regulate the expression of oxidation-related proteins | [7] |
| CoQ10, idebenone | Block lipid peroxidation | [7] |
| Baicalein, PD-146176, AA-861, zileuton | Block lipoxygenase-induced lipid peroxidation | [7] |
| Troglitazone | Specifically inhibits ACSL4 | [12] |
| Zileuton | Specifically inhibits LOX | [113] |
| Vildagliptin, alogliptin, linagliptin | Block DPP4-mediated lipid peroxidation | [7,49] |
| Iron | Deferoxamine, cyclipirox, deferiprone | Deplete iron and prevent iron-dependent lipid peroxidation | [7] |
| Nitrogen oxides | Block the Fenton reaction and inhibit the production of hydroxyl radicals | [114] |
| Curcumin | Chelates iron to reduce iron accumulation; activates the Nrf2 signaling pathway | [115] |

ACSL4: Acyl-CoA synthase long-chain family member 4; LOX: Lipoxygenase; ROS: Reactive oxygen species.