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Nutritional and Metabolic Control of Ferroptosis

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Abstract

Ferroptosis is a type of regulated cell death characterized by an excessive lipid peroxidation of cellular membranes caused by the disruption of the antioxidant defense system and/or an imbalanced cellular metabolism. Ferroptosis differentiates from other forms of regulated cell death in that several metabolic pathways and nutritional aspects, including endogenous antioxidants (such as coenzyme Q_{10}, vitamin E, and di/tetrahydrobiopterin), iron handling, energy sensing, selenium utilization, amino acids, and fatty acids, directly regulate the cells' sensitivity to lipid peroxidation and ferroptosis. As hallmarks of ferroptosis have been documented in a variety of diseases, including neurodegeneration, acute organ injury, and therapy-resistant tumors, the modulation of ferroptosis using pharmacological tools or by metabolic reprogramming holds great potential for the treatment of ferroptosis-associated diseases and cancer therapy. Hence, this review focuses on the regulation of ferroptosis by metabolic and nutritional cues and discusses the potential of nutritional interventions for therapy by targeting ferroptosis.

Contents

1. HISTORICAL VIEW OF FERROPTOSIS: BEFORE AND AFTER THE COINING OF THE TERM FERROPTOSIS

Cell death is an essential process for diverse aspects of the life of multicellular organisms including embryogenesis, tissue homeostasis, and disease development. Moreover, it is intricately intertwined with various other critical biological processes such as immune response and metabolic signaling. In striking contrast with accidental cell death—a biologically uncontrolled demise of

cells exposed to lethal physical, chemical, or mechanical stressors—regulated cell death, including apoptosis, necroptosis, and pyroptosis, is executed by stringently regulated and highly structured signaling cascades, as well as defined molecular effector mechanisms (70). Ferroptosis is a type of nonapoptotic regulated cell death characterized by an iron-dependent, excessive (phospho)lipid peroxidation that is caused by a severely perturbed antioxidant defense system and an aberrant cellular metabolism. Ferroptosis can be suppressed by blocking lipid peroxidation directly or indirectly via pharmacological or genetic means (51). Emerging evidence implicates ferroptosis in various pathologies, including acute organ injury and neurodegenerative diseases, as well as in promoting tumor suppression (98). Therefore, pharmacological modulation of ferroptosis through its induction or inhibition holds great potential for the treatment of ferroptosis-associated diseases and therapy for certain cancer states that are linked to an increased sensitivity to lipid peroxidation (98). Although originally studied in mammalian systems (51), ferroptosis-like cell death has also been observed in evolutionarily more remote species, such as plants, protozoa, and fungi (20, 50, 169). Meanwhile, ferroptosis is increasingly being recognized as one of the most widespread and earliest observed forms of cell death. Ferroptosis is impacted by numerous cellular nutrients and metabolic processes, including endogenous antioxidants [such as coenzyme Q_{10} (CoQ_{10}), vitamin E, and di/tetrahydrobiopterin], iron handling, selenium utilization, and metabolism of amino acids, lipids, and glucose, and also is subject to a number of signaling pathways.

Although the term ferroptosis was coined only quite recently in 2012 as a nonapoptotic form of cell death marked by impaired cystine (the oxidized dimeric form of cysteine) uptake into cells, glutathione (GSH) depletion, and iron-dependent lipid peroxidation (51), ferroptosis-like features and forms of cell death that strongly resemble ferroptosis were documented long before the term was introduced. Such observations include a type of oxidative-stress-induced cell death in neuronal cells termed oxytosis (86, 183). Even earlier, during the 1950s and 1960s, Harry Eagle demonstrated that cysteine deprivation induces cell death (57) and that endogenous synthesis of cysteine can render cells resistant to cysteine-deprivation-induced cell death (58). Now we know that the availability of cysteine is the rate-limiting step in the biosynthesis of GSH, the most abundant reductant in mammalian cells. In the 1970s, investigators found that cystine starvation induces cellular GSH starvation and the accumulation of reactive oxygen species (ROS), efficiently causing cell death (11). Notably, this type of cell death could be rescued by the addition of lipophilic antioxidants, such as vitamin E, without restoring GSH levels (11). In the 1980s, glutathione peroxidase 4 (GPX4), which is nowadays considered as the master regulator of ferroptosis due to its role in eliminating toxic lipid hydroperoxides directly in lipid bilayers, was isolated and purified (195). GPX4 was first described as a peroxidation-inhibiting protein, and several lines of evidence support the unique role of GPX4 in protecting cells against the lethal effects of lipid peroxidation and oxidative stress (23, 185, 194). In the 2000s, an understanding of lipid peroxidation–dependent nonapoptotic cell death caused by the inducible loss of GPX4 in genetically engineered mouse embryonic fibroblasts (164) as well as in hippocampal neurons was achieved and identified as a yet-unrecognized distinct nonapoptotic cell death modality. Through the detailed characterization of the lethal mechanisms of erastin and (1*S*,3*R*)-RSL3 (RSL3), which were previously identified by small molecule screening to induce a similar form of nonapoptotic cell death (51, 218), the term ferroptosis was eventually coined in 2012 (51). On the mechanistic level, studies have shown that erastin and RSL3 trigger this iron-dependent cell death modality by inhibiting cystine import and GPX4, respectively, and established that the cyst(e)ine/GSH/GPX4 axis is the prime defense system against ferroptosis.

During the following decade, several metabolic and nutritional processes were identified as regulators of ferroptosis. Through the discovery of the role of the cyst(e)ine/GSH/GPX4

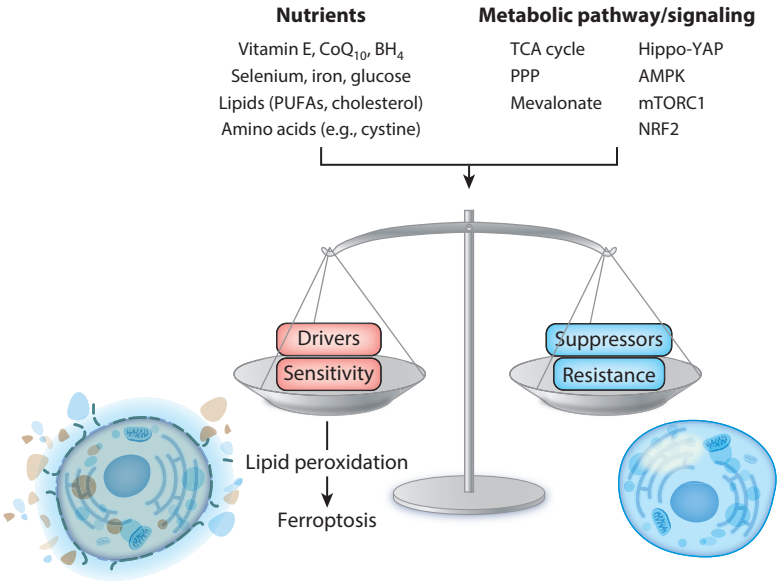

Figure 1

An overview of ferroptosis regulation by nutrients and metabolic pathway/signaling. Abbreviations: AMPK, AMP-activated protein kinase; BH_4, tetrahydrobiopterin; CoQ_{10}, coenzyme Q_{10}; mTORC1, mammalian target of rapamycin complex 1; NRF2, nuclear factor erythroid 2-related factor 2; PPP, pentose phosphate pathway; PUFA, polyunsaturated fatty acid; TCA, tricarboxylic acid; YAP, Yes-associated protein.

pathway in suppressing ferroptosis, the role of phospholipid hydroperoxides (PLOOHs)—a type of lipid-based ROS—as the executioners of ferroptosis have been firmly established. More recently, other ferroptosis surveillance pathways such as ferroptosis suppressor protein 1 (FSP1), GTP cyclohydrolase 1 (GCH1), and dihydroorotate dehydrogenase (DHODH) have been identified (16, 54, 107, 135). Furthermore, the mechanisms of PLOOH synthesis, particularly the synthesis and activation of polyunsaturated fatty acids (PUFAs), the precursor of PLOOHs, have been extensively investigated in the context of ferroptosis. Importantly, all of these studies converge on cellular metabolism and nutrient signaling and suggest a close link between ferroptosis and metabolic processes. Here, we provide a comprehensive review of the regulatory mechanisms of ferroptosis, with special focus on the cellular nutritional and metabolic aspects impacting ferroptosis sensitivity (**Figure 1**).

2. CYST(E)INE/GSH/GPX4: A CENTRAL AXIS MODULATING FERROPTOSIS

2.1. Cysteine and GSH

The hallmark of ferroptosis is the excessive and uncontrolled occurrence of cellular PLOOHs, which can be induced by disrupting the glutathione-dependent and glutathione-independent antioxidant defense systems. The discovery of the importance of sufficient cystine supply via the cystine/glutamate transporter (i.e., system x_c^-), and GSH synthesis for the optimal functioning of GPX4 to prevent lipid peroxidation, established that the cyst(e)ine/GSH/GPX4 axis is the core defense system suppressing ferroptosis (**Figure 2*a***).

The amino acid cysteine and its oxidized dimeric form cystine are crucial to maintain cellular redox homeostasis, mainly through their role in the biosynthesis of GSH, for which cysteine is the rate-limiting substrate. System x_c^- is a heterodimeric plasma membrane cystine/glutamate antiporter that is composed of the transporter protein SLC7A11 (xCT) and transmembrane regulatory protein SLC3A2 (160). Most cellular cystine is imported through system x_c^-, which exchanges extracellular cystine for intracellular glutamate, while some de novo cysteine biosynthesis can occur through the transsulfuration pathway from methionine (83). Imported cystine is converted to its reduced form cysteine via GSH-mediated or thioredoxin reductase 1 (TXNRD1)–mediated reduction (134), which is then used for GSH biosynthesis through the enzymatic machinery including γ-glutamylcysteine synthetase (γ-GCS) and glutathione synthetase (129). GSH is the principal reductant in mammalian cells and is the preferred substrate required for full GPX4 activity. Thus, the depletion of GSH directly leads to the loss of GPX4

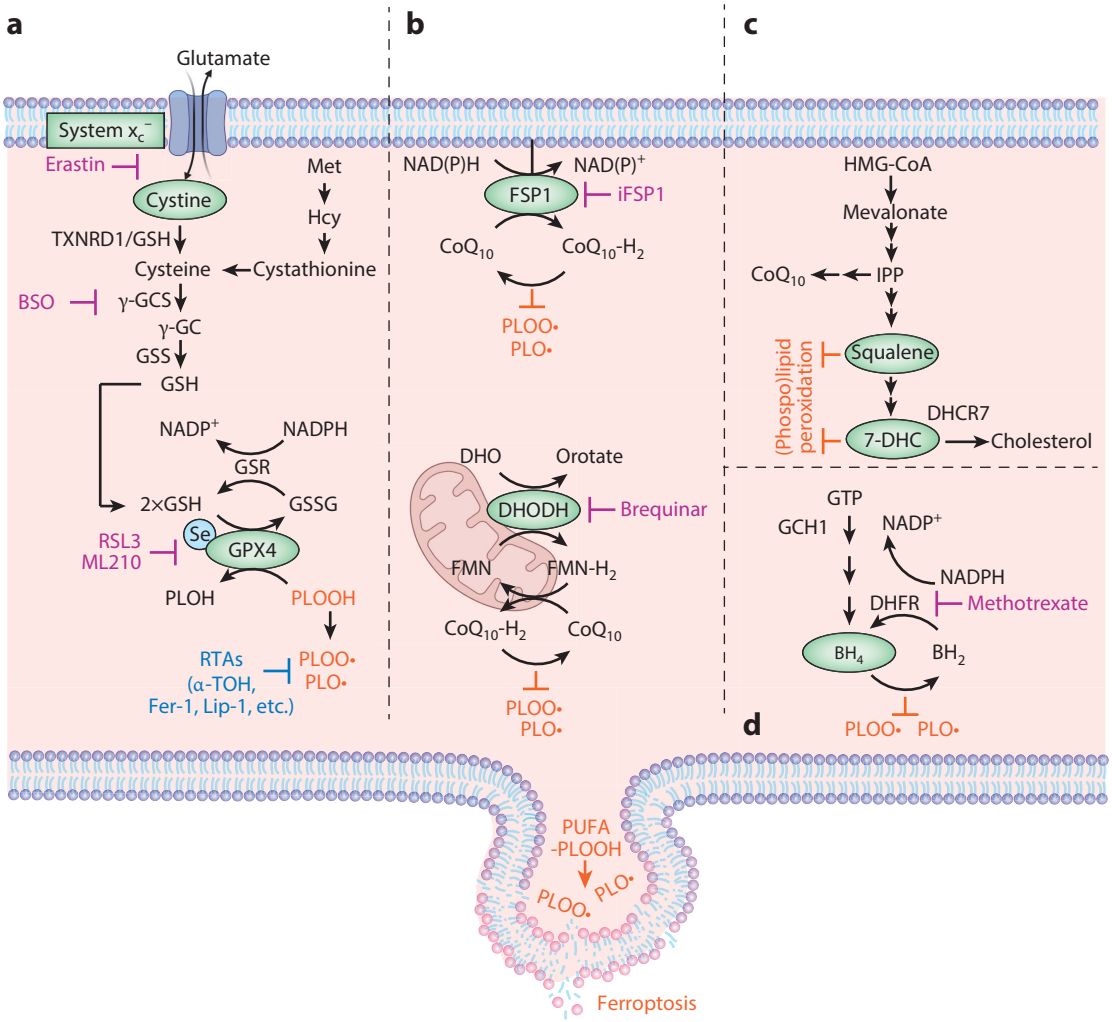

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Figure 2 (*Figure appears on preceding page*)

Major ferroptosis-suppressing pathways. (*a*) Cyst(e)ine/GSH/GPX4 axis. Cystine, taken up via system x_c^-, is reduced to cysteine by GSH or TXNRD1. GSH is synthesized from cysteine through two consecutive reactions involving γ-GC and GSS. GSH is used for the GPX4-mediated reduction of PLOOHs, yielding the corresponding PLOH. Oxidized GSH (GSSG) is recycled by GSR, consuming electrons provided by NADPH. To some extent, cysteine can be provided through the transsulfuration pathway using Met as the substrate. Erastin, BSO, RSL3, and ML210 induce ferroptosis by blocking the step as indicated. RTAs include α-TOH, Fer-1, and Lip-1. (*b*) FSP1- and DHODH-mediated CoQ_{10}-reducing pathways. FSP1, anchored via an N-terminal myristoylation tag in lipid bilayers, suppresses ferroptosis in a glutathione-independent manner by reducing ubiquinone (CoQ_{10}) to ubiquinol (CoQ_{10}-H_2). CoQ_{10}-H_2 in turn suppresses phospholipid peroxidation of lipid bilayers by inhibition of lipid radical-mediated autoxidation, initiated by $PLOO^•$ and $PLO^•$. Located in the mitochondrial inner membrane, DHODH oxidizes DHO to orotate, thereby transferring electrons to CoQ_{10}, yielding CoQ_{10}-H_2. (*c*) Antiferroptotic metabolites produced in the mevalonate/cholesterol synthesis pathway. CoQ_{10}, squalene, and 7-DHC play roles in blocking phospholipid peroxidation. (*d*) GCH-BH_4 pathway. BH_4, generated by the GCH1 pathway, is an endogenous RTA that protects lipid membranes from autoxidation, alone or in synergy with α-TOH. DHFR catalyzes the regeneration of BH_4 from BH_2, whereas inhibition of DHFR by methotrexate sensitizes cells toward ferroptosis. Abbreviations: 7-DHC, 7-dehydrocholesterol; α-TOH, α-tocopherol; γ-GC, γ-glutamylcysteine; γ-GCS, γ-glutamylcysteine synthetase; BH_2, dihydrobiopterin; BH_4, tetrahydrobiopterin; BSO, *L*-buthionine sulfoximine; CoQ_{10}, coenzyme Q_{10}; DHCR7, 7-dehydrocholesterol reductase; DHFR, dihydrofolate reductase; DHO, dihydroorotate; DHODH, dihydroorotate dehydrogenase; Fer-1, ferrostatin-1; FMN, flavin mononucleotide; FMN-H_2, reduced flavin mononucleotide; FSP1, ferroptosis suppressor protein 1; GCH1, GTP cyclohydrolase 1; GPX4, glutathione peroxidase 4; GSH, glutathione; GSS, glutathione synthetase; GSSG, glutathione disulfide; GSR, glutathione-disulfide reductase; GTP, guanosine triphosphate; Hcy, homocysteine; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IPP, isopentenyl pyrophosphate; Lip-1, liproxstatin-1; Met, methionine; NADPH, nicotinamide adenine dinucleotide phosphate; $PLO^•$, phospholipid alkoxyl radical; PLOH, phospholipid alcohol; $PLOO^•$, phospholipid peroxyl radical; PLOOH, phospholipid hydroperoxide; PUFA, polyunsaturated fatty acid; RSL3, (1*S*,3*R*)-RSL3; RTA, radical-trapping antioxidant; Se, selenium; TXNRD1, thioredoxin reductase 1. Figure adapted from images created with BioRender.com.

activity, thereby rendering the cells more susceptible to ferroptosis. Moreover, the reductive microenvironment that is maintained by the system x_c^- is required for cellular selenium uptake and the biosynthesis of GPX4 (150). Erastin, an inhibitor of the system x_c^-, irreversibly blocks cystine import (161), leading to GSH depletion and consequently inducing ferroptosis (51). The lethal effect of erastin can be reversed by β-mercaptoethanol, which bypasses the need for system x_c^- by forming mixed disulfides with cystine that can be imported into the cell by a different transporter (94). Thereby, it also releases one molecule of extracellular cysteine, which can be taken up by neutral amino acid transporters.

2.2. GPX4

GPX4 is the only major enzyme catalyzing the reduction of potentially toxic PLOOHs (195) to nontoxic phospholipid alcohols (PLOHs)—therefore, GPX4 is considered as the guardian of ferroptosis (**Figure 2*a***). The reduction of PLOOH to PLOH by GPX4 requires two electrons, which are provided by GSH, or even by cysteine and other low molecular thiol–containing compounds, and also by protein thiols, particularly when GSH levels are low (133). Currently, pharmaceutical inhibition and genetic knockout of *GPX4* are used as the classic approaches to trigger ferroptosis in cells and mice. Due to its high reactivity toward selenocysteine residues, RSL3 inhibits most selenoproteins including GPX4 and induces ferroptosis by causing the accumulation of PLOOHs, leading to unrepairable damage of membranes, and by releasing toxic lipid peroxidation by-products such as aldehydes (67, 164). The global knockout of *Gpx4* in mice causes early embryonic lethality (219). Tamoxifen-induced *Gpx4* deletion in the whole body, except the brain, causes acute kidney injury and associated lethality, indicating a *Gpx4*-regulated ferroptotic machinery in the kidney proximal tubule (68). The conditional knockout of *Gpx4* in neurons or forebrain neurons leads to neurodegeneration and behavioral dysfunction (34, 164, 207). Many other cell types are also affected by *Gpx4* conditional knockouts that cause cell death

or functional defects in target organs, including $CD4^+$ and $CD8^+$ T cells (137), endothelial cells (209), hematopoietic cells (28), photoreceptor cells (192), and spermatogonia (39). In humans, loss-of-function mutations in the *GPX4* gene cause Sedaghatian-type spondylometaphyseal dysplasia, which is a lethal autosomal recessive disorder that is characterized mainly by skeletal dysplasia combined with cardiac and brain anomalies (172); however, the involvement of lipid peroxidation and ferroptosis in its pathophysiology remains unclear.

3. NUTRITIONAL SUPPRESSORS OF FERROPTOSIS

Biological processes that modulate ferroptosis-promoting or -inhibiting molecules, redox and iron homeostasis, and cellular metabolism can affect ferroptosis. Several metabolites and nutrients have been reported to regulate lipid peroxidation and ferroptosis. Thus far, the effects of nutrients and metabolites that are associated with ferroptosis regulation are classified into the following three categories: (*a*) suppressors and drivers of ferroptosis [e.g., selenium, vitamin E, CoQ_{10}, iron, di/tetrahydrobiopterin, squalene, 7-dehydrocholesterol (7-DHC), and amino acids]; (*b*) fatty acid composition in phospholipids [e.g., PUFAs and monounsaturated fatty acids (MUFAs)]; and (*c*) regulation of ferroptosis pathways.

3.1. Selenium

The trace element selenium, discovered in 1817 and named after the goddess of the moon Selene, is a critical component of selenoproteins, a small and unique family of proteins, including GPX4. Selenoproteins are characterized by the presence of the rare amino acid selenocysteine (Sec) in their polypeptide chain. In the early 1950s, researchers reported the suppression of lipid peroxidation by selenium as well as cysteine (17) and the tissue-protective effect of selenium supplementation in a rat model of liver necrosis based on vitamin E deficiency (now considered as ferroptosis) (163). As GPX4 is one of 25 selenoproteins in humans (109), its biosynthesis and expression are regulated by cellular selenium availability and cotranslational incorporation of Sec (42, 92). Selenium supplementation leads to increased GPX4 expression and subsequent resistance to ferroptosis. Sodium selenite supplementation in the culture medium upregulates GPX4 expression in cultured cells (164), and brain-penetrant selenopeptide administration drives GPX4 expression, which protects against tissue damage in a mouse model with intracerebral hemorrhage (3). The essential role of Sec in GPX4 was recently reported by demonstrating that mice containing cysteine in place of Sec in a GPX4 active site are highly susceptible to hydroperoxide-induced ferroptosis due to irreversible peroxide-mediated overoxidation of the active site cysteine (92). Homozygous *Gpx4*-cysteine mutant mice die near the preweaning stage because of the development of epileptic seizures that are associated with the loss of a critical population of cortical inhibitory interneurons (92). Tamoxifen-inducible *Gpx4*-cysteine–expressing mutant mice are highly sensitized to tubular ferroptosis in kidneys in response to transient ischemia/reperfusion injury (IRI) (187). These findings reveal a specific and indispensable role of selenium in suppressing ferroptosis induced by hydroperoxides. The synthesis and incorporation of Sec in proteins requires a complex machinery (42), suggesting that the genes that are related to the machinery may affect expression of functional GPX4 and ferroptosis. Disruption of selenium incorporation by knockout of the Sec-tRNA gene, which is a specific transfer RNA for Sec incorporation, leads to embryonic death in mice at almost the same stage as that of *Gpx4* knockout mice (22). In a genome-wide CRISPR screening of human-induced pluripotent stem cell–derived neurons, genes responsible for Sec incorporation into proteins (including *PSTK*, *SEPHS2*, and *SEPSECS*) as well as *GPX4* were identified as essential for neurons to survive under proferroptotic oxidative stress (186).

3.2. Vitamin E

Vitamin E is nature's most potent lipophilic radical-trapping antioxidant (RTA) that protects against detrimental lipid peroxidation and ferroptosis (40, 93). Vitamin E scavenges lipid peroxyl radicals to disrupt the propagation phase by forming a vitamin E radical. Dietary vitamin E comprises eight natural forms: α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol, all of which break the autoxidation chain reaction. Supplementation with vitamin E is an effective way to prevent ferroptosis and related diseases, at least in animal models. Vitamin E has been repeatedly shown to rescue certain tissues, including liver, endothelium, and cells such as $CD8^+$ T cells and hematopoietic stem cells, from the deleterious consequences that are induced by tissue-specific disruption of GPX4 (4, 31, 137, 209). In some cells, GSH and thioredoxin systems maintain endogenous α-tocopherol in a reduced state and prevent lipid peroxidation (138). α-Tocopherol hydroquinone, the reduced form of α-tocopherol, has been reported as the most powerful naturally occurring inhibitor of ferroptosis (85); this implication is also highlighted by the fact that sustained vitamin E deficiency causes neurodegeneration (193). The neurological phenotype of vitamin E deficiency is similar to Friedreich's ataxia, which is a genetic neurological disorder whose pathogenesis is reported to be linked to ferroptosis (111), suggesting that endogenous vitamin E exerts a powerful preventive role against neuronal ferroptosis.

3.3. Coenzyme Q_{10} and Its Reducing Systems FSP1 and DHODH

CoQ_{10}, also known as ubiquinone, is an endogenously produced lipophilic antioxidant that has been shown to prevent the harmful oxidation of lipids and proteins (202). In mitochondria, CoQ_{10} is essential for electron transfer through the electron transport chain, thereby maintaining the mitochondrial membrane potential and promoting ATP synthesis. However, CoQ_{10} not only resides in mitochondria but is also present elsewhere in the cell in almost all of the lipid membranes—hence its name ubiquinone (202). The precise role of extramitochondrial CoQ_{10} remained unclear for many decades after its discovery. Independent genome-wide screens have helped to discover the cell-intrinsic pathways that protect against ferroptosis and revealed that the extramitochondrial CoQ_{10} antioxidant system serves as a robust ferroptosis surveillance mechanism (**Figure 2*b***).

This GPX4-independent ferroptosis suppression mechanism involves FSP1, previously called AIFM2 (16, 54), which is mainly localized in cell membrane structures, including the plasma membrane, Golgi apparatus, and lipid droplets. FSP1 suppresses lipid peroxidation and ferroptosis through its NAD(P)H:ubiquinone oxidoreductase activity (60). By consuming NAD(P)H, FSP1 reduces extramitochondrial ubiquinone (CoQ_{10}) to its reduced form ubiquinol ($CoQ_{10}H_2$), which acts as a lipophilic RTA to directly reduce lipid radicals in membranes or indirectly by regenerating oxidized α-tocopheryl radicals (**Figure 2*b***). This protective role elucidates the long-standing mystery of why some cells and tissues indeed contain a large pool of extramitochondrial CoQ_{10}. Additional studies demonstrated that the loss of this defense system by *FSP1* deletion impaired the growth of tumors lacking GPX4 expression (16) and exacerbated kidney damage following ischemia/reperfusion (187). FSP1 belongs to the family of type II NADH:quinone oxidoreductases and catalyzes the two-electron transfer from NAD(P)H to quinones without any energy-generating site; this catalyzation is the same reaction as mitochondrial complex I but without proton pumping (140). Perhaps this is analogous to the role of FSP1 in brown adipose tissue, where it is implicated in cold- and diet-induced thermogenesis (148).

In addition to FSP1, DHODH has recently been reported as a defense mechanism against ferroptosis related to CoQ_{10} reduction (135) (**Figure 2*b***). DHODH is an iron-containing flavin-dependent enzyme that plays an essential role in the de novo synthesis of pyrimidines by catalyzing

the oxidation of dihydroorotate to orotate; orotate is then converted to uridine monophosphate, the RNA nucleotide involved in ribosome biogenesis (61). In the mitochondrial inner membrane, DHODH inhibits ferroptosis by reducing ubiquinone to ubiquinol, which is ultimately coupled with the oxidation of dihydroorotate to orotate to detoxify lipid peroxides that accumulate in the mitochondria. While inhibition of DHODH alone does not induce ferroptosis, it was shown to markedly sensitize cells to ferroptosis (135).

The identification of FSP1 and DHODH as suppressors of ferroptosis suggests that increased CoQ_{10} synthesis might efficiently protect cells from ferroptosis. In this regard, the mevalonate pathway, in which CoQ_{10}, cholesterol, 7-DHC, squalene, and isopentenyl pyrophosphate (IPP) are generated, also affects ferroptosis. The ferroptosis inducer FIN56 deprives CoQ_{10} in cells (171). Statins, inhibitors of the mevalonate pathway and commonly used as cholesterol-lowering drugs, have been shown to sensitize cells to ferroptosis (196). In addition, IPP, the precursor of CoQ_{10}, is a limiting substrate for the enzymatic isopentenylation of Sec-tRNA (66), which is essential for the incorporation of Sec.

3.4. Squalene and 7-DHC

Among the metabolites of the cholesterol synthesis pathway, squalene and 7-DHC have been reported to exert antiferroptotic activity (**Figure 2*c***). Squalene, a lipophilic metabolite accumulating in cell membranes and lipid droplets, has antioxidant activity, and thereby protects against ferroptosis (75). Accumulation of squalene by genetic deletion of squalene monooxygenase, catalyzing the oxidation of squalene to 2,3-oxidosqualene in the cholesterol synthesis pathway, leads to ferroptosis resistance in cancer cells. Most recently, 7-DHC was reported as a new player as a cell-intrinsic mechanism for suppressing ferroptosis (67). 7-DHC is a precursor of cholesterol and found in abundant quantities in the epidermis. 7-DHC, due to its superior reactivity toward peroxyl radicals, shields phospholipids from autoxidation by acting as a potent radical sink. Intracellular accumulation of 7-DHC induced by loss of function of 7-dehydrocholesterol reductase, the enzyme converting 7-DHC to cholesterol, lowered the basal sensitivity of cells toward ferroptosis in cancer cells (67), thus constituting a potential cell-intrinsic mechanism to evade cancer cell death.

3.5. GCH1-BH_4-DHFR Axis

GCH1, the rate-limiting enzyme for tetrahydrobiopterin (BH_4) synthesis, has been reported to inhibit ferroptosis independent of GPX4 via its metabolic products BH_4 and dihydrobiopterin (BH_2) (**Figure 2*d***) (107, 176). BH_4 and BH_2 act as endogenous RTAs and are regenerated by dihydrofolate reductase (DHFR). In addition to its role as an RTA, BH_4 may promote the synthesis of CoQ_{10} by converting phenylalanine into tyrosine, which can be further converted to 4-OH-benzoate, a precursor of CoQ_{10} (107).

3.6. Gas Transmitters: NO and H_2S

Nitrogen oxide (NO) is a highly reactive gaseous radical mediator with antioxidant properties that terminates lipid peroxidation reactions and was recently reported to exert a cytoprotective action against ferroptotic stimuli (87). Accordingly, NO donor compounds protect against ferroptosis by aborting the lipid peroxidation chain reaction (87), and expression of inducible nitric oxide synthase was reported to modulate ferroptosis susceptibility (102). Hydrogen sulfide (H_2S), a gas transmitter widely present in various tissues and organs, regulates oxidative stress and also suppresses ferroptosis (105, 204).

4. METABOLITES DRIVING FERROPTOSIS

4.1. Fatty Acid Metabolism: The Balance Between PUFAs and MUFAs

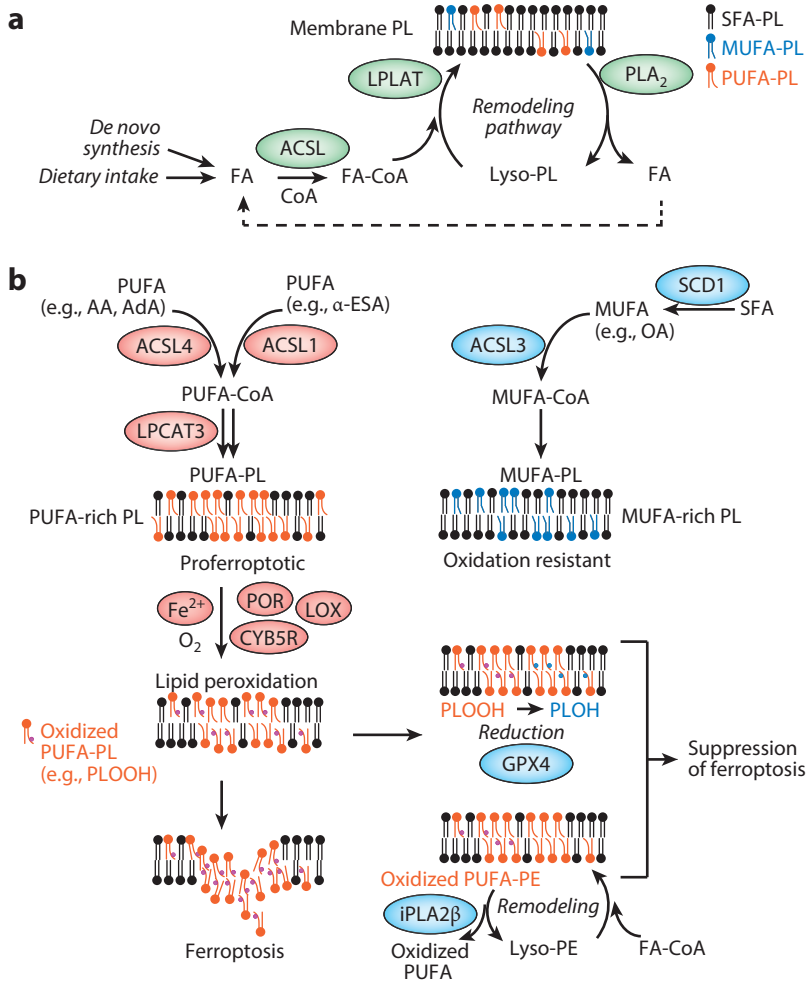
The unrestrained generation of PUFA-containing phospholipid peroxides in cellular membranes is the ultimate trigger for ferroptosis (2). Unlike saturated fatty acids and MUFAs, PUFA chains in membrane lipids are far more susceptible to lipid oxidation. Lipid peroxidation is initiated by the removal of a bisallylic hydrogen atom from PUFAs incorporated in phospholipids that constitute membrane lipid bilayers. Hydrogen atom abstraction leads to the formation of a carbon-centered phospholipid radical (PL•). In a subsequent reaction with molecular oxygen, a phospholipid peroxyl radical (PLOO•) is formed (41), which in turn removes hydrogen from another PUFA to form PLOOHs. If not reduced to the corresponding PLOH by GPX4, lipid-derived radicals—in particular, PLOO•—react with PUFA phospholipids (PUFA-PLs) to trigger the lipid peroxidation chain reaction by further abstracting hydrogen atoms, reacting with oxygen and the formation of PLOOHs. Eventually, this leads to the generation of a variety of secondary lipid peroxidation products, including 4-hydroxynonenal and malondialdehyde, that will oxidize and modify proteins. The extensive oxidation of phospholipid radicals and the generation of lipid peroxidation breakdown products may eventually damage membrane integrity, ultimately rupturing cell membranes (67). Thus, membranes containing high levels of PUFA-PL are generally vulnerable to lipid peroxidation and ferroptosis (98).

PUFAs in mammalian cells are obtained from dietary essential fatty acids or the product of the orchestrated action of desaturases and elongases (**Figure 3*a***). Fatty acids, including PUFAs, are acylated with coenzyme A (CoA) by their respective acyl-CoA synthetase long-chain family member (ACSL) to form fatty acid–CoA, which is subsequently esterified into phospholipids by lysophospholipid acyltransferase (131). Fatty acids of phospholipids are liberated by phospholipase A_2 (PLA_2) to yield free fatty acids and lysophospholipids (lyso-PLs). Lyso-PLs are converted to phospholipids in the presence of fatty acid–CoA in the remodeling pathway. This biosynthesis and remodeling of phospholipids modulate the composition of the lipid profile in the cell membrane (**Figure 3*a***).

Phosphatidylethanolamines (PEs) bearing arachidonic acid (C20:4) and adrenic acid (C22:4) were initially identified as the main substrates for lipid peroxidation in the context of ferroptosis (100), although more recent data suggest that a wide range of PUFAs can be involved (54). Genome-wide genetic screens identified ACSL4 as a central regulator of cell sensitivity to ferroptosis (52, 55). ACSL4 preferentially ligates long-chain PUFAs, including arachidonic acid and adrenic acid, with CoA. These products can then be re-esterified and incorporated into phospholipids by lysophosphatidylcholine acyltransferase 3 (LPCAT3), thereby increasing the incorporation of long-chain PUFAs into lipids and membranes, increasing the risk of lipid peroxidation (**Figure 3*b***). Thus, the loss of ACSL4 causes a shift from long-chain PUFA tails to MUFA tails in phospholipids (55, 100), rendering ACSL4-deficient cells highly resistant to ferroptosis. Similarly, the pharmacological blocking of ACSL4 by rosiglitazone or other thiazolidinediones has been shown to prevent lipid peroxidation and ferroptosis (55). In fact, ACSL4 expression levels correlate with cell sensitivity to ferroptosis inducers (55, 196); thus, the suppression of ACSL4 expression may be a principal mechanism in increasing the cells' resistance to ferroptosis (25, 210).

As PUFA-rich lipid membranes are susceptible to ferroptosis, treatment of cells with PUFAs promotes ferroptosis. Treatment with dihomo-γ-linolenic acid (20:3n-6), a kind of PUFA, induces ferroptosis in human cancer cells and *Caenorhabditis elegans* (153), akin to the treatment of cells with PUFA-PL hydroperoxide [e.g., PC (16:0_18:2 (9Z,11E));13OOH] causing sensitization to ferroptosis in human cancer cells and rat cardiomyoblasts (142, 181). In addition, PUFAs, in particular arachidonic acid, can induce inflammatory bowel disease by triggering ferroptosis in

Gpx4-deficient mice (139). Omega-3 (n-3) and n-6 PUFAs are able to induce ferroptosis in cancer cells under ambient acidosis, which prompts the cancer cells to take up exogenous fatty acids (48). Consistently, a diet rich in n-3 long-chain PUFAs (such as docosahexaenoic acid) delayed the growth of tumors in mice when compared with mice fed a MUFA-enriched diet (48), indicating dietary PUFAs as an emerging potential adjuvant antitumor modality. Conjugated linoleic acids (another class of PUFAs), such as α-eleostearic acid, that are acylated and incorporated into cellular phospholipids in an ACSL1-dependent manner promote lipid peroxidation and ferroptosis (13) (**Figure 3*b***). The oral administration of tung oil, which is naturally rich in α-eleostearic acid, promotes ferroptosis and suppresses tumor growth in mice (13). Alternatively, the displacement of PUFAs from membrane phospholipids by MUFAs is an intrinsic way to prevent lipid peroxidation (**Figure 3*b***). MUFA biosynthesis requires stearoyl-CoA desaturase 1 (SCD1), which converts saturated fatty acids into MUFAs (152), and ACSL3 preferentially catalyzes the acylation of MUFAs (132). Indeed, previous studies have demonstrated that exogenous supplementation of MUFAs and promotion of SCD1-mediated MUFA production suppress ferroptosis (132, 184, 217). Melanoma



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

The phospholipid makeup dictates ferroptosis sensitivity. (*a*) Synthesis and remodeling of membrane PLs. ACSL catalyzes the ATP-dependent ligation of FAs with CoA to produce FA-CoA, which is esterified to PLs by LPLAT. FAs esterified in PLs are liberated by PLA_2 to generate free FAs and lyso-PLs. Lyso-PLs are converted to PLs in the presence of FA-CoA by LPLAT in the remodeling pathway. (*b*) AA and AdA are preferably acylated by ACSL4, while conjugated-linoleic acids, such as α-ESA, are acylated by ACSL1. Acylated AA and AdA (PUFA-CoA) are incorporated into PLs by LPCAT3 or other transferases. PUFA-PLs are oxidized nonenzymatically or enzymatically with a potential involvement of LOX, cytochrome POR, and CYB5R. Accumulation of oxidized PUFA-PLs eventually triggers ferroptosis, likely involving reactive aldehydes downstream of lipid peroxidation. Reduction of PLOOHs to PLOHs by GPX4 and remodeling of oxidized PLs (especially, hydrolysis of hydroperoxyeicosatetraenoic acid by $iPLA_2\beta$) suppress lipid peroxidation and ferroptosis. SFAs are converted to MUFAs by SCD1. MUFAs, such as OA, are acylated by ACSL3 and are incorporated into PLs. PUFA-rich PLs are prone to lipid oxidation and proferroptotic, while, in contrast, MUFA-rich PLs are resistant to oxidation. Abbreviations: α-ESA, α-eleostearic acid; AA, arachidonic acid; ACSL, acyl-CoA synthetase long-chain family member; AdA, adrenic acid; CoA, coenzyme A; CYB5R, cytochrome b5 reductase; FA, fatty acid; GPX4, glutathione peroxidase 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; LOX, lipoxygenase; LPLAT, lysophospholipid acyltransferase; lyso-PL, lysophospholipid; MUFA, monounsaturated fatty acid; OA, oleic acid; PE, phosphatidylethanolamine; PLA_2, phospholipase A_2; PL, phospholipid; PLOH, phospholipid alcohol; PLOOH, phospholipid hydroperoxide; PUFA, polyunsaturated fatty acid; POR, cytochrome P450 oxidoreductase; SCD1, stearoyl-CoA desaturase 1; SFA, saturated fatty acid.

cells in the lymph have also been found to evade ferroptosis by enriching phospholipids with oleic acid (a MUFA) in an ACSL3-dependent manner (191).

In addition to the reduction of PLOOHs by GPX4, elimination of oxidized PUFAs from phospholipids is an alternative way to suppress ferroptosis (**Figure 3*b***). Oxidatively modified PUFA-PLs, especially hydroperoxyeicosatetraenoic acid PE (HpETE-PE), have been reported as a characteristic proferroptotic signal in ferroptosis (100). A member of the Ca^{2+}-independent family of PLA_2 enzymes ($iPLA_2$), $iPLA_2\beta$, has been shown to act as an antiferroptotic regulator by eliminating the proferroptotic signal HpETE-PE in phospholipids by metabolizing to lyso-PLs and oxidized fatty acids during membrane remodeling (14, 32, 179). In addition, other enzymes involved in the fatty acid metabolism have also been reported as regulators of ferroptosis. For example, LPCAT3 (52), fatty acid desaturase-2 (197), acetyl-CoA carboxylase (171), and 2,4-dienoyl-CoA reductase (18) have been reported as drivers of ferroptosis, while lysophosphatidylserine lipase (103) has been reported as a suppressor of ferroptosis. Collectively, the profile of phospholipids containing sufficient PUFAs in the membrane determines cell sensitivity to ferroptosis, indicating that the manipulation of PUFA synthesis or degradation can modulate cell susceptibility to ferroptosis (2).

4.2. Peroxisome and Plasmalogens

In addition to ACSL4-dependent PUFA-PLs, peroxisome-dependent polyunsaturated ether phospholipids have been reported to be susceptible to triggering ferroptosis when oxidized (43, 231). Accordingly, depletion of several genes involved in peroxisome biogenesis, such as *PEX3*, *PEX10*, and *PEX12*, decreased the number of peroxisomes and lowered ferroptosis sensitivity in cancer cells with diminished production of PUFA-ether phospholipids, such as plasmalogens. Unlike common phospholipids, plasmalogens contain an ether instead of an ester bond. At the *sn*-1 position, they contain a nonhydrolyzable chain that is ether linked, while the chain at *sn*-2 is linked by a conventional ester. Due to this special chemical structure, the synthesis of plasmalogen is initiated in the peroxisome. A ferroptosis-resistant phenotype, similar to that observed in ACSL4-deficient cells, was observed by depleting peroxisomal enzymes, such as alkylglycerone phosphate synthase, fatty acyl-CoA reductase 1, and glycerone phosphate O-acyltransferase, that are involved in the synthesis of plasmalogens (231). These findings suggest that peroxisomes have

an impact on cell sensitivity to ferroptosis by affecting the synthesis of plasmalogens, a subclass of ether phospholipids.

4.3. Contribution of Enzymatic Lipid Peroxidation Pathway to Ferroptosis

Although it is well accepted that the degree of unsaturation of lipid bilayers determines the sensitivity of cells to ferroptosis, it remains obscure what the trigger of lipid peroxidation as the prime event in ferroptosis induction is. Two possibilities of enzymatic and nonenzymatic routes are constantly being discussed. Lipid peroxidation can be initiated nonenzymatically by the direct generation of lipid radicals due to physical and chemical stresses or indirectly via the help of the hydroxyl radical (•OH), one of the most reactive forms of ROS, from H_2O_2 in a reaction known as the Fenton reaction involving iron as the catalyst (154). During enzyme-mediated initiation, certain lipoxygenases (LOXs) can directly oxygenate PUFAs in membranes, leading to the formation of PLOOH, which in turn may trigger lipid peroxidation and ferroptosis as described in the foregoing (217). This possibility is supported by the observation that some pharmacological LOX inhibitors can prevent ferroptosis (122). In addition, another study reported that binding of a small scaffold protein, phosphatidylethanolamine-binding protein 1, seemingly promotes ferroptosis by enabling LOXs to generate lipid peroxides (206). However, frequently used LOX inhibitors possess intrinsic RTA activity and can prevent ferroptosis by nonspecifically suppressing lipid peroxidation (40, 165). In addition, deletion of *Alox15* (the gene encoding 15-lipoxygenase) failed to prevent ferroptosis induced by loss of GPX4 in cultured cells and in several animal models (68, 137), suggesting that LOXs are not the actual drivers of lipid peroxidation and that alternative mechanisms may compensate for the loss of LOX activity. For instance, cytochrome P450 oxidoreductase (POR) has been recently reported to be involved in enzymatic lipid peroxidation triggering ferroptosis (232). POR is a flavin reductase required for the electron transfer from nicotinamide adenine dinucleotide phosphate (NADPH) to cytochrome P450 (CYP) and is thus essential for CYP-mediated reactions in the metabolism of drugs, xenobiotics, and steroid hormones. POR facilitates lipid peroxidation by transferring electrons from NAD(P)H to oxygen to generate H_2O_2, which reacts with iron to produce hydroxyl radials for peroxidation of PUFA-PLs (214) and/or by cycling between ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions in the heme component of CYPs (12), thus promoting ferroptosis. Similarly, NADH–cytochrome b5 reductase has been reported to regulate ferroptosis by promoting lipid peroxidation in a mechanism similar to that of POR (214).

4.4. Iron Handling in Ferroptosis

As the name ferroptosis implies, iron is an important element in ferroptosis. Intracellular redox-active iron promotes ferroptosis by catalyzing the formation of lipid radicals as described in the foregoing. Accordingly, iron chelators prevent ferroptosis (at least to some extent) (51), whereas iron loading into tumor cells by nanoparticles has been repeatedly shown to promote ferroptosis (104, 200). This strongly suggests that nonenzymatic, iron-dependent Fenton reaction is crucial for ferroptosis (40). PLOOHs can react with both Fe^{2+} and Fe^{3+} to generate lipid radicals, driving the lipid peroxidation chain reaction; however, at least for the initiation phase of this iron-catalyzed reaction, Fe^{2+} (in the labile iron pool) is likely more relevant.

Consequently, many cellular processes that affect the import, export, storage, and release of cellular labile iron alter cell sensitivity to ferroptosis (228) (**Figure 4**). Extracellular ferric ions complexed with transferrin are taken up into cells mainly through transferrin receptor 1 (TFR1), which internalizes transferrin-bound iron into cells (6). Thus, knockdown of TFR1 lowers iron uptake into cells and thereby protects against ferroptosis (73). Intriguingly, the expression of TFR1

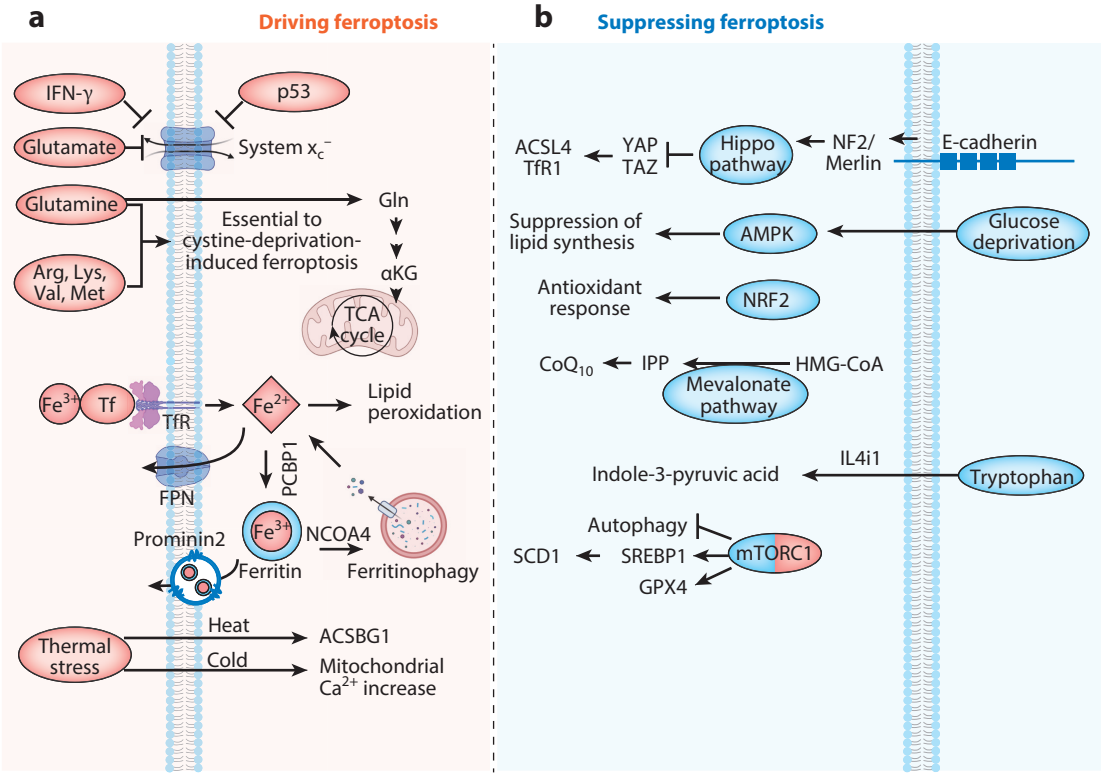


Figure 4

Metabolic regulation of ferroptosis. The metabolic pathways driving and suppressing ferroptosis are summarized. (*a*) IFN-γ and p53 sensitize cells to ferroptosis by suppression of system x_c^- expression. Glutamate competitively blocks cystine uptake through system x_c^-. Deprivation of glutamine, arginine, lysine, valine, or methionine independently suppresses ferroptosis induced by erastin or cystine withdrawal. The import, export, storage, and release of cellular labile iron alter cell sensitivity to ferroptosis. Heat stress augments lipid peroxidation through the upregulation of ACSBG1. Cold stress induces ferroptosis with involvement of mitochondrial Ca^{2+} increase. (*b*) Activation of e-cadherin-mediated Hippo pathway, activation of AMPK by glucose starvation, and oxidative stress response mediated by NRF2 suppress ferroptosis. Mevalonate-pathway-generating CoQ_{10} affects ferroptosis. Tryptophan-derived indole derivatives, such as indole-3-pyruvic acid, confers antiferroptotic functions. Inhibition of mTOR suppresses erastin-induced ferroptosis, whereas it sensitizes cells to ferroptosis induced by GPX4 inhibition, such as by RSL3. Abbreviations: αKG, α-ketoglutarate; ACSBG1, acyl-CoA synthetase bubblegum family member 1; ACSL4, acyl-CoA synthetase long-chain family member 4; AMPK, AMP-activated protein kinase; CoQ_{10}, coenzyme Q_{10}; Fe^{2+}, ferrous ion; Fe^{3+}, ferric ion; FPN, ferroportin; GPX4, glutathione peroxidase 4; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IFN-γ, interferon gamma; IL4i1, interleukin 4 induced 1; IPP, isopentenyl pyrophosphate; mTORC1, mammalian target of rapamycin complex 1; NCOA4, nuclear receptor coactivator 4; NF2, neurofibromin 2; NRF2, nuclear factor erythroid 2-related factor 2; PCBP1, poly(rC)-binding protein 1; RSL3, (1*S*,3*R*)-RSL3; SCD1, stearoyl-CoA desaturase 1; SREBP1, sterol regulatory element binding transcription factor 1; TAZ, transcriptional coactivator with PDZ-binding motif; TCA, tricarboxylic acid; Tf, transferrin; TfR, transferrin receptor; YAP, Yes-associated protein. Figure adapted from images created with BioRender.com.

has been reported as a ferroptosis marker, and antibodies against TFR1 may detect ferroptosis in cell culture and tissue contexts (64). After ferric iron is taken into cells, Fe^{3+} is converted to the highly reactive Fe^{2+} and released from endosomes into the cytoplasm. This is mediated by divalent metal transporter 1 (DMT1), thereby forming an unstable iron pool in the cytoplasm. A fraction of Fe^{2+} in the iron pool is stored as ferritin to protect cells from toxic iron-mediated damage. Downregulating ferritin expression, especially its heavy chain, increases the labile iron pool and accordingly sensitizes cells to ferroptosis (146). Specifically, knockdown of the ferritin

heavy chain gene, *Fth1*, promotes cardiomyopathy, likely by enhancing ferroptosis (62), whereas knockdown of DMT1 has also been shown to suppress ferroptosis (174). Poly(rC)-binding protein 1 (PCBP1) is a cytosolic iron chaperone that assists in the delivery of iron to ferritin and other nonheme iron proteins (21). Hepatocyte-specific knockout of *Pcbp1* in mice causes increased labile iron and thereby exacerbates ferroptosis-associated liver damage (155). The iron chaperone frataxin has also been implicated as a regulator of ferroptosis susceptibility (56). Increasing cellular iron availability by ferritinophagy, an autophagic mechanism specifically degrading ferritin, likewise promotes ferroptosis (72, 89). Thus, blockage of autophagy or the knockdown of the selective cargo receptor nuclear receptor coactivator 4 limits ferritin degradation and confers resistance to ferroptosis. Conversely, mechanisms that enhance cellular iron export render cells more resistant to ferroptosis (24, 190). The ferrous iron exporter ferroportin 1 (FPN1) is responsible for cellular iron export and coupled with multicopper ferroxidases, including ceruloplasmin (6). Consequently, decreased expression of FPN1 (78) or ceruloplasmin (166) increases the sensitivity of cells to ferroptosis by inhibiting iron export outside cells. Alternatively, iron can be released in the form of ferritin-containing multivesicular bodies; thus, prominin2, which drives this process, suppresses ferroptosis sensitivity (24). Under ferroptotic stress, the expression of prominin2 is reported to be stimulated by 4-hydroxynonenal, a lipid peroxidation breakdown product, leading to intrinsic ferroptosis resistance (26). Iron-regulatory proteins IRP1 and IRP2, which are central in the posttranscriptional regulation of genes involved in intracellular iron homeostasis, indirectly impact on ferroptosis by regulating downstream iron metabolism proteins, including TFR1, FPN1, and FTH1 (220). The transcription factor BTB domain and CNC homologue 1, a regulator of heme and iron metabolism, promotes ferroptosis by repressing the transcription of *Fth1* and *Ftl1*, as well as glutathione synthesis enzymes (149). Liberating iron from heme via heme oxygenase 1 (HO-1)-mediated heme degradation has also been implicated in ferroptosis; however, the respective data remain contradictory, suggesting either ferroptosis-promoting or -suppressing roles of HO-1 in the death process (110, 180). Therefore, the role of HO-1 in ferroptosis is still obscure and requires further investigations.

5. METABOLIC MODULATION FOR FERROPTOSIS

Mounting evidence implicates multiple metabolic signaling pathways in the modulation of the cells' susceptibility to ferroptosis (**Figure 4**).

5.1. Amino Acids as Proferroptotic Nutrients

The amino acids glutamate and glutamine play important roles in ferroptosis induction. Glutamate-induced oxicytotoxicity in HT-22 neuronal cells, previously called oxytosis (183), was shown to be involved in ferroptosis. High concentrations of extracellular glutamate competitively block cystine uptake through system x_c^-, leading to cellular GSH exhaustion and the accumulation of lipid hydroperoxides (51) and cell death. This type of cell death is iron dependent and is prevented by radical trapping of ferroptosis inhibitors (51). These features thus share the concept of ferroptosis.

In the absence of glutamine in the culture medium, cystine starvation and blockage of cystine import by erastin fails to induce ferroptosis (73). The observation that α-ketoglutarate (73), a product of glutaminolysis, can replace the requirement of glutamine for ferroptosis (73) suggested the importance of glutaminolysis in ferroptosis. Mechanistically, the promotion of lipid peroxidation by an increased generation of mitochondria-derived ROS was considered (74). However, glutamine is not necessary for ferroptosis induced by RSL3 (an inhibitor of GPX4) (74). Deprivation of other several amino acids (arginine, lysine, valine, and methionine) as well as glutamine

independently suppressed ferroptosis induced by erastin2 (an analog of erastin) or cystine cowithdrawal (38). Pyruvate (>5 mM) can also replace the requirement of glutamine for erastin-induced ferroptosis (198). Taken together, strongly reduced proliferation rates of cells following (acute) perturbation of amino acids homeostasis is a likely mechanism to suppress ferroptosis under conditions of disrupted cystine utilization.

Metabolic pathways involving other amino acids also impinge on ferroptosis susceptibility in various ways. Interleukin 4 induced 1, an amino acid oxidase secreted from immune cells, generates indole-3-pyruvic acid from tryptophan, which confers antiferroptotic functions by its RTA activity and through the activation of an oxidative stress response (223). Other indole derivatives also have RTA activity (84), and gut microbiota can produce a large amount of indole from tryptophan (141, 175). It is thus tempting to hypothesize that microbiota-derived indoles might exert their action to prevent lipid peroxidation and ferroptosis in the intestine. Knockdown or inhibition of aspartate aminotransaminase triggered ferroptosis by enhancing labile iron availability through the autophagy pathway activated by repression of mitochondrial metabolism and by promoting a catabolic state (108). Intriguingly, unlike in mouse embryonic fibroblasts, deficiency of the cystine transporter xCT does not induce ferroptosis in macrophages (106). Mechanistically, elevated expression of carnosine dipeptidase II, a cytosolic dipeptidase that hydrolyses cysteinylglycine dipeptide, can recruit cystine into cells under cystine-deprived conditions in xCT knockout macrophages, thereby protecting cells against ferroptosis (106).

5.2. Glucose

Energy stress depletes ATP, increases ROS levels, and induces cell death. For example, glucose limitation (1 mM of glucose in culture medium) induces nonferroptotic cell death, such as in cancer cells overexpressing xCT (125); in contrast, glucose starvation (glucose-free culture medium) blocks ferroptosis (115, 118). This protective effect of glucose starvation was found to depend on the activity of AMP-activated protein kinase (AMPK), a sensor of cellular energy status (115). When glucose is depleted, AMPK is activated, turning on an energy stress-protective program against ferroptosis through acetyl-CoA carboxylase and PUFA biosynthesis. Cancer cells with high basal AMPK activation are resistant to ferroptosis, and AMPK inactivation sensitizes these cells to ferroptosis, demonstrating that energy stress inhibits ferroptosis partly through AMPK-mediated energy stress signaling (115). In contrast, a high-glucose environment enhances sensitivity of cells toward ferroptosis induced by x_c^- inhibitors in cancer cells (173). Mechanistically, SLC2A1 (GLUT1)-mediated glucose uptake promotes glycolysis and facilitates pyruvate oxidation, thus fueling the tricarboxylic acid cycle and stimulating fatty acid synthesis, which eventually facilitates lipid peroxidation-dependent ferroptotic death (173). These findings suggest that metabolic rewiring overcomes ferroptosis resistance.

5.3. NADPH

Cells constantly need to maintain NADPH levels to sustain redox homeostasis and cell survival in addition to multiple catabolic processes. A key role for NADPH in the prevention of ferroptosis is to nourish the GSH- and FSP1-dependent systems, as well as to maintain mevalonate biosynthesis and elongation of fatty acids. NADPH can be produced through a variety of metabolic pathways, including the pentose phosphate pathway (PPP) (126). Cellular NADPH abundance is considered as a biomarker for predicting sensitivity to ferroptosis across several cancer cell lines (170). Cancer cells with elevated expression of system x_c^- require high NADPH supply provided by the PPP route to reduce highly insoluble cystine to the more soluble cysteine. In these cells, glucose limitation restricts glycolytic flow to the PPP, thereby depleting cellular NADPH levels.

This in turn results in marked accumulation of intracellular cystine, leading to rapid cell death (125). Cellular NADPH levels are also regulated by metazoan SpoT homologue 1 (MESH1), a cytosolic NADPH phosphatase, which degrades NADPH into NADH. Thus, overexpression of MESH1 depletes cellular NADPH and sensitizes cells to ferroptosis (49). NADPH can also be synthesized through phosphorylation of NAD by NAD kinase (NADK). Hence, suppressing NADK also decreases intracellular NADPH levels and thereby sensitizes the cells to ferroptosis (170).

5.4. mTORC1

Mammalian target of rapamycin complex 1 (mTORC1) is a nutrient sensor that is activated by amino acids (especially leucine and arginine) (208), energy (e.g., glucose), and growth factors (e.g., insulin). Activation of mTORC1 signaling promotes protein biosynthesis and suppresses autophagy. Several studies have reported conflicting findings on the role of mTOR signaling in both the pro- and antiferroptotic pathway (117). A compound screening study identified that several ATP-competitive mTOR inhibitors suppress ferroptosis triggered by system x_c^- inhibition or direct cystine deprivation in a targeted manner (38). It was also demonstrated that knockdown of mTORC1 suppresses ferroptosis induced by system x_c^- inhibition, suggesting that mTORC1 signaling is a proferroptotic pathway. In contrast, other studies reported that the pharmacological inhibition of mTORC1 decreased GPX4 expression, thus sensitizing cancer cells to ferroptosis (226). Similarly, inhibition of the PI3K-AKT-mTOR signaling axis increases sensitivity of cells to ferroptosis (222). The activation of mTORC1 has also been shown to induce the expression of SREBP1 (a transcription factor regulating lipid metabolism) and its transcriptional target SCD1, mediating ferroptosis-suppressing activity by increasing cellular MUFA content (222). Furthermore, mTOR inhibition and RSL3 synergistically induce autophagy-dependent ferroptosis (127). These results therefore indicate that mTOR signaling acts in suppressing ferroptosis. Similar to those in HO-1, the effects of mTOR signaling in ferroptosis differ substantially and might depend on the type of cells and ferroptosis inducers (such as inhibitors of x_c^- or those of GPX4) used; as such, these effects must be investigated further.

5.5. NRF2: Antioxidant Response

Nuclear factor erythroid 2-related factor 2 (NRF2) is a master transcription factor of the cellular antioxidant response, controlling the expression of genes that counteract oxidative and electrophilic stresses. Many NRF2 downstream target genes are involved in preventing or correcting cellular redox imbalances. Consequently, the oxidative stress response by NRF2 can mitigate ferroptosis by stimulating the expression of several of its canonical target genes (5). With the noble exception of *GPX4*, almost all gene products that are implicated in ferroptosis regulation are transcriptionally regulated by NRF2, including genes involved in glutathione regulation (e.g., *SLC7A11*, both subunits of γ-GCS), NADPH regeneration (e.g., *G6PD* and *PGD*), iron regulation (e.g., ferritin and *FPN*), and the recently described antiferroptotic players *FSP1/AIFM2* and *DHFR* (36, 53, 143). Consequently, NRF2 activation, genetically or pharmacologically, such as by bardoxolone methyl, renders cells resistant to ferroptosis (69, 180). Of note, some conventional NRF2 activators, such as natural flavonoids (e.g., kaempferol and quercetin), possess direct RTA activity in addition to their NRF2 stimulatory activity (221); this dual activity thus leaves an inherent issue in considering the antiferroptotic action of these compounds, as is the case for many LOX inhibitors (as described above), which were shown to have potent RTA activity.

5.6. Hippo-YAP Signaling

The Hippo-YAP pathway, which might be modulated by nutrient availability and cell metabolism, is involved in coordinating organ growth and homeostasis (90). The observation that cells grown at high density are more resistant to ferroptosis led to the investigation of the role of the Hippo-YAP pathway in ferroptosis (210). This cell density effect is perhaps analogous to earlier data, when it was shown that c-Myc-driven B cell lymphoma cells (e.g., Burkitt's lymphoma) rapidly grow under high cell densities, whereas they readily die when plated at nonpermissive cell culture concentrations (23). Moreover, *Gpx4* knockout cells can easily survive when these cells are plated at high cell concentrations and grown to confluence (164). Concerning the underlying mechanisms, cell density-mediated intercellular interactions play a role in the regulation of ferroptosis. Cell–cell contact activates e-cadherin-mediated Hippo signaling depends on intracellular NF2/merlin, which in turn impairs activation of the transcriptional coregulator YAP (216). Since YAP targets several crucial ferroptosis modulators, such as ACSL4, TFR1, and possibly others, Hippo activation and YAP inactivation render cells more resistant to ferroptosis (**Figure 4**). Similarly, TAZ, a homologue of YAP, also regulates cell-density-dependent ferroptosis in cancer cells that primarily express TAZ instead of YAP (216). These findings provide mechanistic insights into why cancer cells with mesenchymal or metastatic properties may show increased susceptibility to ferroptosis (196).

5.7. Thermal Stress in Ferroptosis: Heat and Cold Stress

Changes in extracellular temperature affect a series of cellular responses, including metabolic state; indeed, both hyperthermic and hypothermic stress induce ferroptosis. In plants, heat stress (55°C, 10 min) has been shown to trigger ferroptosis-like cell death characterized by iron dependency, GSH depletion, and lipid ROS increase (44, 50); however, this type of cell death requires Ca^{2+} influx unlike in ferroptosis of mammalian cells. Heat stress also facilitates the induction of ferroptosis in mammalian cells and could be a valid strategy in cancer therapy. Metabolic control by heat (45°C) augments iron oxide nanoparticle-induced oxidative stress, triggers lipid peroxidation through the downregulation of glutathione biosynthesis enzymes and upregulation of acyl-CoA synthetase bubblegum family member 1, and thereby causes ferroptosis (211). In the process of heat-induced ferroptosis, the direct effect of heat alone may promote lipid peroxidation, as lipids such as oils naturally undergo oxidation under heat (157). Likewise, severe cold stress also induces ferroptosis (82). Cultured cells treated with prolonged cold stress by placing plates on ice induced cell death that was prevented by radical trapping ferroptosis inhibitors and iron chelators. Activation of the ASK1-p38-mediated stress signaling pathway and the involvement of mitochondrial Ca^{2+} have been reported in this type of death (147). Resistance to cold-induced ferroptosis may explain why mammalian hibernators can endure severe prolonged hypothermia that is lethal to nonhibernators, including humans and mice. A mammalian hibernator, e.g., the Syrian hamster, has a phospholipid composition in liver that is less susceptible to peroxidation than that in mice, and it has superior ability to retain dietary α-tocopherol in the body (7). Indeed, hepatocytes from Syrian hamsters exhibited resistance to prolonged cold culture, whereas murine hepatocytes underwent cold-induced ferroptosis.

In a clinical setting, cold-stress-induced tissue damage is observed during organ preservation for transplantation, and preservation solution supplemented with iron chelators has been shown to result in improved tissue function of the transplanted organ (114). Human livers retrieved for organ transplantation can be stored at −4°C by supercooling, effectively extending the ex vivo life of the organ with a reduced number of TdT-mediated dUTP-biotin nick end labeling–positive dead cells compared with standard hypothermic preservation without ice (47). These findings

suggest the involvement of ferroptosis in cold-stress-induced damage of transplantation organs and that supercooling might prevent them from succumbing to ferroptosis due to deeper metabolic stasis.

6. SUPPRESSION AND INDUCTION OF FERROPTOSIS

6.1. Suppressors of Ferroptosis

There is a growing interest in the identification and development of new chemical entities as novel ferroptosis suppressors and inducers (see comprehensive review in 123). In principle, ferroptosis can be prevented by the following approaches: (*a*) scavenging lipid hydroperoxyl radicals with RTAs, (*b*) depleting iron, (*c*) decreasing PUFA-containing PLs, and (*d*) modulating ferroptosis-regulating pathways.

Considering that ferroptosis is driven by phospholipid peroxidation, administration of lipophilic RTAs, such as ferrostatin-1 (51), liproxstatin-1 (68), and vitamin E, is a key strategy for preventing ferroptosis (230). These agents and their analogs are highly effective in cell models of ferroptosis and can be effective in some in vivo contexts. In addition, compound screening revealed that drugs with potential RTA activity, including some US Food and Drug Administration–approved drugs [such as rifampicin (an antibiotic), promethazine (an antihistamine), and bazedoxifene (a drug used for osteoporosis)], prevent ferroptosis (38, 142). However, the cellular localization of the respective RTA required for effective suppression of ferroptosis has not been fully elucidated. Ferrostatin-1 and its analogs (ferrostatins) localize to lysosomes, mitochondria, and the endoplasmic reticulum with little accumulation in the nucleus and plasma membrane. Nonetheless, accumulation in neither lysosome nor mitochondria is required for ferrostatin-mediated suppression of ferroptosis (77). Therefore, identification of the functional localization of the RTAs may help to reveal the primary cellular site of lipid peroxidation triggering ferroptosis and the precise role of the respective organelle involved in ferroptosis. Another strategy for suppressing ferroptosis is depletion of the cellular labile iron pool by using iron chelators, such as deferoxamine, or using lysosome/autophagy inhibitors (bafilomycin A1 and chloroquine) to block ferritinophagy (72). A third approach is to administer deuterated PUFAs that are chemically more resistant to peroxidation due to the isotope effect (230). Such deuterated PUFAs retard the radical chain reaction of lipid peroxidation and prevent ferroptosis (217). Similarly, administration of MUFAs has been shown to limit ferroptosis and may be potentially exploited for therapeutic applications (132). A fourth strategy involves targeting enzymes responsible for ferroptosis regulation, such as the upregulation of the ferroptosis-suppressing genes, including *GPX4*, *FSP1*, *DHODH*, and *NRF2*, and the downregulation of the ferroptosis-promoting genes, including *ACSL4* and *POR*.

6.2. Inducers of Ferroptosis

Increasing the cell's sensitivity to ferroptosis can be achieved by the following approaches: (*a*) blocking the cyst(e)ine/GSH/GPX4 axis, (*b*) facilitating iron-mediated oxygen radical formation, (*c*) increasing the intracellular labile iron pool, (*d*) increasing PUFA-containing phospholipids, and (*e*) modulating ferroptosis-regulating pathways.

Erastin and its analog imidazole ketone erastin (113), with increased potency and water solubility, are strong and irreversible inhibitors of system x_c^-. Sulfasalazine also targets system x_c^-, but with lower potency and selectivity. Sorafenib has been repeatedly reported to induce ferroptosis, possibly through the inhibition of system x_c^- (112); however, a recent report has shown that the cell death–inducing activity of sorafenib is independent of system x_c^- and that it fails to engage ferroptosis across many cancer cell lines (229). *L*-buthionine sulfoximine is a specific inhibitor

of γ-GCS that causes GSH depletion and ferroptotic cell death (142). RSL3 is the prototypical GPX4 inhibitor (218), but it also inhibits other selenoproteins due to the lack of specificity among selenoproteins (71). Although RSL3 is the most frequently used ferroptosis inducer for in vitro experiments, it is difficult to use it for in vivo experiments because of its poor bioavailability. Akin to RSL3, ML210, a nitroisoxazole-containing compound, also covalently inhibits GPX4 and induces ferroptosis (205), and ML210 exhibits improved selectivity toward GPX4 (59). FIN56 induces ferroptosis via a dual mechanism, promoting the degradation of GPX4 and affecting the CoQ_{10} biosynthesis pathway (171). Ferroptocide, a derivative of the natural product pleuromutilin, induces ferroptosis by inhibition of thioredoxins. Organic endoperoxide compounds, such as artemisinin and artesunate, can induce ferroptosis by generating iron-dependent free radicals (151). Artemisinin is a naturally occurring antimalarial compound discovered from traditional Chinese medicine (189). The cytotoxic potency of artemisinin has been explored for its potential anticancer property (9). FINO2, an endoperoxide-containing 1,2-dioxolane, discovered by screening the derivatives of artemisinin (1), is a unique ferroptotic inducer that functions by direct oxidation of iron and indirect inactivation of GPX4 (76). Other artemisinin derivatives, such as dihydroartemisinin, can induce lysosomal degradation of ferritin in an autophagy-independent manner, increasing the cellular labile iron level and thereby sensitizing cells to ferroptosis (33).

Nanoparticle-based strategies to deliver iron or PUFAs to kill tumor cells by inducing ferroptosis have also been tested. Biocompatible iron oxide nanoparticles can serve as iron ion suppliers to both enhance ROS production and participate in iron metabolism involved in ferroptosis (156). The addition of PUFAs to nanoparticles has been reported to improve the therapeutic efficacy of anticancer agents (158).

As ferroptotic cell death is regulated by multiple metabolic pathways, a multidimensional approach of combining several proferroptotic features might efficiently trigger ferroptosis. Pharmacological inhibition of FSP1, DHODH, and DHFR by iFSP1, brequinar, and methotrexate, respectively, has been shown to sensitize cells to ferroptosis in synergy with inhibitors of GPX4 or system x_c^- (54, 135, 176).

7. POTENTIAL PHYSIOLOGICAL ROLE OF FERROPTOSIS

Evolutionary, ferroptosis may be (one of) the earliest forms of regulated cell death (27). However, the physiological relevance of ferroptosis remains unclear, unlike that of other forms of regulated cell death, such as apoptosis and necroptosis. However, indirect evidence suggests a possible physiological role for ferroptosis in innate immune surveillance and the eradication of tumors and pathogens. The finding that $CD8^+$ T cell–derived interferon-γ sensitizes tumor target cells to ferroptosis by suppression of system x_c^- expression (159, 201) suggests a role for ferroptosis in the innate immune response. Furthermore, it provides insights into investigating cells that exploit ferroptosis for disease prevention or the shaping of organs during development. The potential function of ferroptosis in innate responses is not limited to mammals and may be relevant in bacteria and plants as well. The prokaryotic bacterium *Pseudomonas aeruginosa* takes advantage of theft-ferroptosis as a virulence mechanism using its LOX. Targeting and hijacking the host redox lipid remodeling pathway leads to the accumulation of proferroptotic PLOOHs in human bronchial epithelial cells (45, 46). In rice, induction of ferroptosis-like cell death prevents infection by the fungus *Magnaporthe oryzae* by removing infected cells and preventing the pathogen from spreading (169). In addition, a ferroptosis inducer, artemisinin (151), which is a generator of iron-dependent free radicals, is used as medicine for malaria parasite (19). These findings support the hypothesis that pathogen eradication in the host analogous to sensitizing tumor cells for T cell–mediated killing may be a beneficial physiological function of ferroptosis.

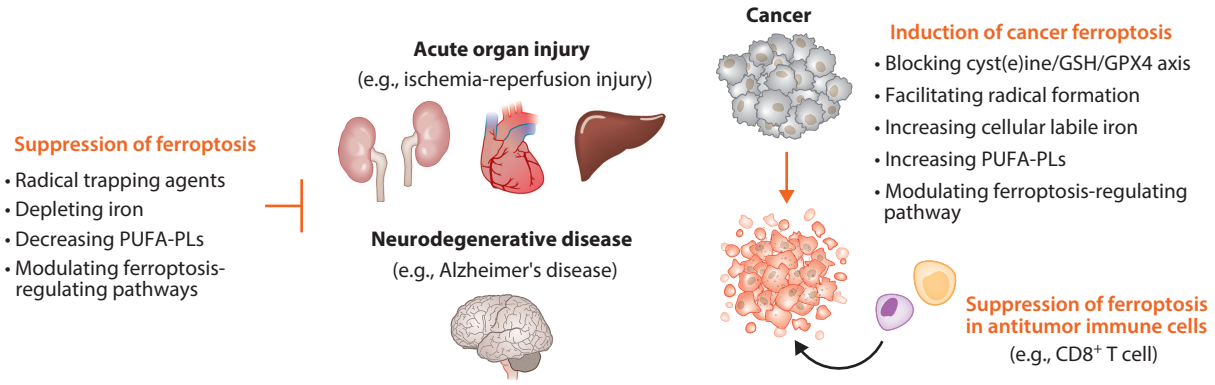


Figure 5

Contribution of ferroptosis to different disease contexts and therapeutic strategies targeting ferroptosis. Ferroptosis contributes to the pathophysiology of acute organ injury, neurodegenerative diseases, and cellular mechanisms in tumor suppression of therapy-resistant cancers. Suppression of ferroptosis in organ injury and neurodegenerative diseases, induction of ferroptosis in cancer cells, and prevention of ferroptosis in antitumor cells are considered as therapeutic strategies for each disease condition. Abbreviations: GSH, glutathione; GPX4, glutathione peroxidase 4; PUFA-PL, polyunsaturated fatty acid phospholipids.

8. IMPLICATIONS OF FERROPTOSIS IN DISEASE

Although the actual contribution of ferroptosis to physiology remains unclear, its role in various disease contexts has been extensively investigated (**Figure 5**). In addition, the pharmacological targeting of ferroptosis has been repeatedly reported to be a valid strategy for the treatment of these conditions in various animal models.

8.1. Ferroptosis Sensitivity in Cancer

Ferroptosis has been linked to cancer, and numerous cancer-related genes and signaling pathways indeed regulate ferroptosis. Compelling evidence suggests that targeting genes and pathways that regulate ferroptosis may provide new opportunities for treating cancers. For example, p53, a key transcription factor suppressing cancer development, was reported to inhibit cystine uptake by repressing the expression of system x_c^-, thus rendering cells more sensitive to ferroptosis and thereby suppressing tumor growth (97). Intriguingly, therapy-resistant mesenchymal cancer cells (196) and drug-tolerant persister cells (81) have been shown to become highly dependent on GPX4 for their survival, thus possibly presenting a druggable vulnerability for ferroptosis induction. For instance, the cell differentiation state has been negatively correlated with the sensitivity to ferroptosis inducers (188), and system x_c^- was shown to be required for the metastasis of cancer cells (162). Thus, induction of ferroptosis might be regarded as a promising therapeutic strategy for the treatment of therapy-resistant cancers.

Blocking the endogenous defense systems that regulate ferroptosis is an effective way to induce ferroptosis in cancer cells. Inhibiting system x_c^- either pharmacologically (227) or genetically (10, 162) has been shown to restrain tumor growth and metastasis in various types of cancer in mouse models. When the x_c^--dependent cystine import is blocked by erastin, increased expression of the oxidative stress sensor protein DJ-1 (also known as Parkinson disease protein 7) allows cells to maintain cysteine levels provided by the transsulfuration pathway (29). Thus, suppression of DJ-1 synergistically enhanced the antitumor efficacy of erastin analog in vivo. Targeting GPX4 is also a promising therapeutic strategy to sensitize cancer cells toward ferroptosis and to eradicate tumors. Indeed, genetic ablation of *GPX4* in cancer cells prevented tumor growth in various xenograft

animal models (196, 233). However, GPX4 lacks suitable binding pockets, and this deficiency poses a major challenge for conventional medicinal chemistry approaches to develop targeted drug therapy (42), likely requiring other targeting strategies. The widely used GPX4 inhibitors, including RSL3, are strong electrophiles that irreversibly modify the catalytic Sec residue within selenoproteins, leading to the irreversible inhibition of almost all human selenoproteins including GPX4 (71). Thus, this class of inhibitors is likely not ideal for in vivo application primarily due to its lack of specificity in addition to metabolic stability issues in plasma. The CoQ_{10}-related ferroptosis defense may be a suitable target for inducing ferroptosis in cancer cells. FSP1 is abundantly expressed in most tumor cell lines, and genetic deletion or pharmacological inhibition of FSP1 by iFSP1 treatment robustly sensitizes cells to RSL3-induced ferroptosis in a number of cancer cells (54). The DHODH inhibitor brequinar, in combination with system x_c^- inhibitor sulfasalazine, suppresses tumor growth (135), especially with low expression levels of GPX4, by inducing ferroptosis in a xenograft mouse model.

The involvement of ferroptosis has also been reported in cancer immunotherapy (212). $CD8^+$ T cells play a central role in antitumor immunity, although their activity is subject to inhibition in the tumor microenvironment (99). Immune checkpoint blocking therapy with anti–programmed death ligand 1 antibodies stimulates $CD8^+$ T cells to secrete interferon gamma, which suppresses system x_c^- activity in target cancer cells, sensitizing them to ferroptosis (201). In the tumor microenvironment, fatty acids have been shown to induce ferroptosis in $CD8^+$ T cells in a CD36-dependent manner, thereby dampening the intratumoral $CD8^+$ T cell effector function with an overall impairment of their antitumor activity (130). Consistently, inhibiting ferroptosis of $CD8^+$ T cells by blocking CD36 enhances the antitumor efficacy of immunotherapy by anti–programmed death 1 antibodies. Besides $CD8^+$ T cells, inhibition of ferroptosis might also be crucial for the survival of antitumor immune cells such as B cells and natural killer cells in the tumor microenvironment (212). Therefore, conceptually, immunotherapy in combination with ferroptosis induction in cancer cells and/or ferroptosis suppression in antitumor immune cells would have synergistic anticancer effects (**Figure 5**).

Irradiation can trigger ferroptosis by upregulation of ACSL4 in cancer cells (116); thus, ferroptosis is also implicated as a contributor to some of the adverse events of radiation, such as lung fibrosis and death of granulocyte-macrophage hematopoietic progenitor cells (120, 225). Ferroptosis is also reported to be involved in the cell death mechanism underlying the cytotoxicity of some existing chemotherapeutics such as doxorubicin (63) and cisplatin (142). However, the direct cytotoxic effects of these agents do not involve ferroptosis alone. Cisplatin-induced cell death in cultured cells is insensitive to lipophilic RTAs (142), while doxorubicin activates caspase-3 (182), indicating the contribution of cell death mechanisms other than ferroptosis under in vitro conditions. Nonetheless, in vivo, several cell death pathways may contribute to cytotoxic pathophysiology (37). Thus, ferroptosis is likely part of the complex of cell death pathways involved in cytotoxicity of these drugs.

8.2. Ferroptosis in Ischemia-Reperfusion Injury

Compelling evidence suggests that ferroptosis is the main contributor to cell death associated with IRI (35) (**Figure 5**). Ischemia followed by reperfusion is known to generate ROS. This in turn induces massive cell death and inflammatory responses in the affected organs, resulting in devastating diseases, including brain stroke and ischemic heart disease as well as injuries to the liver and kidney. Ischemia/reperfusion is commonly used as an organ damage model in animals. Ferroptosis inhibitors, mainly lipophilic RTAs, have been successfully applied in animal models of ischemia/reperfusion-related tissue injuries including brain (3), heart (65), intestine (121),

kidney (124), and liver (68). Similarly, ferroptosis inhibitors also ameliorate acute organ damage other than IRI, such as in a genetic model of inducible whole-body *Gpx4* deletion and animal models accompanied by massive cell death (68, 124, 128). In addition, recent single-cell transcriptomic analysis showed that ferroptotic stress (such as downregulation of the genes relating to the cyst(e)ine/GSH/GPX4 axis and upregulation of ACSL4) triggered a persistent proinflammatory state in damaged proximal tubular cells following kidney IRI (91). These findings expand on the roles of ferroptotic stress being a trigger of cell death to include the promotion and accumulation of proinflammatory cells that underlie pathologic inflammation and fibrosis. Ischemia/reperfusion is an inevitable clinical consequence of organ transplantation. Thus, ferroptosis has also been implicated in posttransplant complications in transplantation of the heart (119) and liver (213), and the suppression of ferroptosis is warranted as a therapeutic strategy to overcome IRI in solid organ transplantation.

The iron status in disease conditions, in which ferroptosis is suspected to play a role, has been explored. In an animal model of kidney IRI, exogenous iron infusion was shown to exacerbate kidney damage, while the treatment with iron chelators was tissue protective (167). Iron chelators have also been investigated in animal models of extrarenal acute organ injury, with respect to the heart, lungs, liver, brain, and immune system, and have provided encouraging results (167). The removal of toxic forms of nontransferrin-bound iron from the circulation has been shown to prevent downstream harmful effects caused by nontransferrin-bound iron, such as lipid peroxidation and ferroptosis. Clinically, iron overload indicated by high serum ferritin levels has been associated with hepatic IRI observed in the donor liver of transplantation (213). Feeding mice a high-iron-containing diet, under severe but not mild iron overload, triggered liver damage with lipid peroxidation, which was rescued by the ferroptosis inhibitor ferrostatin-1 (199).

8.3. Role of Ferroptosis in Neurodegenerative Disease

A progressive loss of neuronal cells is the common feature of neurodegenerative diseases. Ferroptotic cell death has been implicated in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) (34, 96) (**Figure 5**). Lipid peroxidation and iron accumulation have been characterized in these diseases long before the discovery and description of ferroptosis (79). Lipofuscin is a highly oxidized cross-linked pathological biomolecule of yellow-brown pigment granules, such as those found in the retina and brain, and appears to be the product of the oxidation of unsaturated fatty acids and contains metals such as iron (145). It is traditionally associated with aging and has a potential physiological role in neurodegenerative disorders. The presence of lipofuscin is indirect evidence of lipid peroxidation in a lesion. Genetic studies in mice confirmed that conditional *Gpx4* deletion can cause symptoms mimicking neurodegeneration, such as motor neuron degeneration (34) and cognitive impairment (80). Furthermore, iron chelators and lipophilic RTAs have been tested to mitigate neurodegeneration (e.g., Alzheimer's disease and Parkinson's disease) in animal models (15, 136). Edaravone, which is a radical scavenger and an approved drug for treatment of ALS and stroke, prevents ferroptosis in vitro (88). Cu^{II} (atsm), an investigational new drug that ameliorates neuronal loss in mouse models of ALS and Parkinson's disease, has been reported to protect against ferroptosis (177). Vatiquinone (EPI-743, α-tocotrienol quinone), possessing antiferroptotic activity (101), has been clinically studied for several mitochondrial diseases presenting neurological deficits as well as Friedreich ataxia (ClinicalTrials.gov: NCT04378075, NCT04577352). The potential benefit of deuterated PUFA administration has been reported in animal models of neurodegeneration, and clinical trials are currently ongoing in patients suffering from neurodegenerative disease conditions, such as ALS (168). So far, a number of small-scale clinical trials potentially targeting

ferroptosis as a therapeutic intervention for neurodegenerative diseases by iron chelators, antioxidants, and selenium supplementation have been performed; however, the beneficial effects of these interventions have been controversial and not very convincing up until now (8).

Owing to the lack of robust biomarkers specific for ferroptosis, the link between ferroptosis and human diseases remains poorly explored and still limited. Our current understanding of this subject is mostly based on studies using conditional *Gpx4* knockout mice and pathological models that recapitulate the features of ferroptosis. To consider the clinical targets for modulating ferroptosis, optimal disease conditions (type of disease, stages during the chronic disease period, severity, genetic background) and corresponding efficacy of the drugs must be carefully considered.

8.4. COVID-19 and Ferroptosis

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic is responsible for a huge number of respiratory syndrome coronavirus disease 2019 (COVID-19) cases, which are characterized by respiratory distress and, in severe cases, cytokine storm, coagulopathy, and multiple organ dysfunction syndrome (30). Several studies reported the potential relationship between ferroptosis and COVID-19 (215). Selenoproteins are implicated in the antioxidant, anti-inflammatory, and antiviral actions of selenium (178), which has been found to be a significant factor affecting the incidence and severity of a number of viral diseases in both animals and humans. Indeed, lower selenium status was associated with poor cure rates and mortality risk from COVID-19 (144, 224). SARS-CoV-2 infection suppresses mRNA expression of *GPX4* in Vero cells (203). In an autopsy case with severe COVID-19, immunohistopathological analysis showed accumulated oxidized phosphatidylcholine in the affected myocardial tissue (95). These findings likely imply ferroptosis as a detrimental factor in organ injury during shock and multiorgan failure in severe COVID-19 cases.

9. CONCLUSION AND FUTURE PERSPECTIVE

Ferroptosis is currently considered to be the consequence of the perturbation of various metabolic pathways and the improper functioning of the main ferroptosis surveillance systems. Recent advances have provided insights into the molecular mechanisms that are involved in ferroptosis, particularly its relationship with cellular metabolism, nutrient signaling, and extracellular redox conditions. Thus, variations in the composition of the culture medium and cell conditions as well as diets provided to animal models greatly influence the cellular metabolic state in cell culture and in vivo and thus inevitably impact the susceptibility of cells and tissues to ferroptosis. In addition, the influence differs from the effects of ferroptosis inducers (such as inhibitors of system x_c^- or those of GPX4). As such, these environmental variabilities (e.g., cell confluency and concentrations of glucose, serum, iron, selenium, and amino acids as well as overall antioxidant content) need to be carefully controlled when investigating the role of ferroptosis in vitro and in vivo. Besides these considerations, there are several outstanding questions in the field of ferroptosis. The precise molecular events, following the accumulation of peroxidation of PUFA-PLs, that lead to ferroptosis are not yet known. Potentially beneficial physiological functions of ferroptosis likewise remain to be uncovered. Moreover, the precise biological markers for detecting ferroptosis need to be identified for facilitating future research. Biomarkers that are unique to ferroptosis—analogous to active caspase-3 for apoptosis or phosphorylated mixed lineage kinase domain like pseudokinase for necroptosis—must be identified and validated in patients suffering from neurodegenerative diseases or other organ damage conditions that are suspected to involve ferroptosis. Biomarker identification will be indispensable not only in determining the involvement of ferroptosis in a pathological condition but also in assessing the pharmacodynamics of novel antiferroptotic

therapies. Establishment of ferroptosis-specific biomarkers will also promote the application of our understanding of ferroptosis into clinical settings for diagnosis and treatment of several difficult-to-treat diseases.

DISCLOSURE STATEMENT

M.C. holds patents for some of the compounds described herein and is a cofounder and shareholder of ROSCUE Therapeutics GmbH.

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