

Review

Ferroptosis: Death by Lipid Peroxidation

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Ferroptosis is a regulated form of cell death driven by loss of activity of the lipid repair enzyme glutathione peroxidase 4 (GPX4) and subsequent accumulation of lipid-based reactive oxygen species (ROS), particularly lipid hydroperoxides. This form of iron-dependent cell death is genetically, biochemically, and morphologically distinct from other cell death modalities, including apoptosis, unregulated necrosis, and necroptosis. Ferroptosis is regulated by specific pathways and is involved in diverse biological contexts. Here we summarize the discovery of ferroptosis, the mechanism of ferroptosis regulation, and its increasingly appreciated relevance to both normal and pathological physiology.

Discovery of Ferroptosis

Cell death is essential for fundamental physiological processes such as development, immunity, and tissue homeostasis; moreover, cell death is often dysregulated in degenerative and neoplastic diseases. Both apoptotic and nonapoptotic cell death modalities have increasingly been necessary to explain diverse biological processes involving cell loss. Two regulated forms of nonapoptotic cell death, necroptosis and ferroptosis, have been shown recently to play significant roles in numerous biological contexts [1,2]. While the mechanisms and physiological relevance of necroptosis have been reviewed recently [3], we focus here on the molecular mechanisms controlling ferroptosis and its relevance to health and disease.

Early life on Earth developed in the absence of oxygen [4]; approximately 2.4 billion years ago, the composition of the atmosphere changed dramatically, probably due to oxygen production from emerging photosynthetic organisms [5,6]. After this Great Oxygenation Event, oxygen in the atmosphere ultimately rose from only trace abundances to its current 21% abundance [5,6]. The emergence of copious amounts of oxygen in the atmosphere was challenging for organisms with membranes having polyunsaturated lipids that contain bis-allylic carbons, because these are highly susceptible to lipid peroxidation in the presence of oxygen [7]. This peroxidation reaction is dramatically accelerated by divalent metals, especially Fe(II) [7].

While life probably originated using saturated amphiphilic lipids (i.e., charged but hydrophobic molecules lacking carbon-carbon double bonds, such as simple fatty acids) [8], before long, monounsaturated and polyunsaturated lipids were integrated into lipid metabolism and membrane biochemistry, as they allow increased tunability of membrane fluidity [9]. However, the presence of these polyunsaturated fatty acids created a liability after the Great Oxygenation Event, especially because of the abundance of Fe(II) and Fe(II)-dependent enzymes: without a means to prevent lipid peroxidation, these membranes became a source of damaging oxidative species as reactive lipid peroxides were generated.

These observations on the early origin and essential functions of polyunsaturated fatty acids, Fe(II)-dependent oxidation chemistry and abundant oxygen suggest the hypothesis that the evolution of defenses against lipid peroxidation was an early selective event in the development

Trends

Ferroptosis is a regulated, nonapoptotic form of cell death distinct from other cell death modalities.

Loss of GPX4 activity and subsequent accumulation of lipid hydroperoxides executes ferroptosis.

Diffuse large B cell lymphomas and renal cell carcinomas are particularly susceptible to GPX4-regulated ferroptosis.

Inhibition of ferroptosis may represent a promising therapeutic approach for treating pathological conditions such as acute kidney injury.

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of life. Over time, this defense system was adapted to a more complex control mechanism that allowed fine-tuned control over lipid peroxidation, enabling it to be harnessed to generate signaling molecules (e.g., in the inflammatory cascade), suppressed to preserve cell integrity under high peroxidation stress (e.g., in neurons or renal tubules), or unleashed to cause lethal lipid peroxidation (e.g., in nascent neoplastic cells).

Ferroptosis is this nonapoptotic, peroxidation-driven form of regulated cell death that requires abundant and accessible cellular iron; the existence of this ancient form of cell death was unknown and discovered only recently using a pharmacological approach [10]. The first ferroptosis-inducing compounds, erastin [11] and RSL3 [12], were discovered using high-throughput screening of small-molecule libraries [11,12]. The mode of cell death induced by these compounds was surprisingly found to be nonapoptotic, as cells treated with erastin and RSL3 died in the absence of apoptotic hallmarks [11–13], and in cells where the core apoptosis machinery – caspases, BAX, and BAK – was suppressed [14]. Although nonapoptotic, erastin-induced cell death proceeds normally on knockdown of RIPK1/RIPK3 or pharmacological inhibition of RIPK1 [10,15] (our unpublished data), a known component of necroptosis [16]. Therefore, the cell death phenotype induced by erastin and RSL3 is distinct from other reported modalities, including apoptosis and necroptosis.

Further studies [13,17] identified lipophilic antioxidants (α -tocopherol, butylated hydroxytoluene, and β -carotene) as strong suppressors of erastin-induced cell death, suggesting that ROS, probably lipophilic in nature, are involved in this cell death process. Analysis with dichlorofluorescein (DCF), a ROS-detecting dye, revealed that erastin causes the generation of ROS in sensitive cell lines [10,13]. Moreover, iron chelators were identified as inhibitors of cell death induction after RSL3 treatment, revealing the requirement for cellular iron [12].

Using modulatory profiling, an unbiased pharmacological and genetic profiling system in which lethal compounds are classified based on their functional profiles [14,18], erastin and RSL3 were found to cluster together, suggesting that they share a similar cell death mechanism. This erastin–RSL3 cluster is distinct from other lethal compounds that induce apoptosis and necrosis. Taking these findings together, the mode of cell death induced by erastin, RSL3, and related compounds was proposed to be a previously unrecognized form of cell death termed ferroptosis [ferro, ‘ferrous ion’ (Fe^{2+}); ptosis, ‘fall’], suggesting a critical role for cellular iron in this regulated form of oxidative cell death [10].

Mechanism of Ferroptosis Induction by Erastin and RSL3

Key regulators of ferroptosis have been discovered through characterizing the mechanism of action of erastin and RSL3 using multipronged approaches (Figure 1, Key Figure).

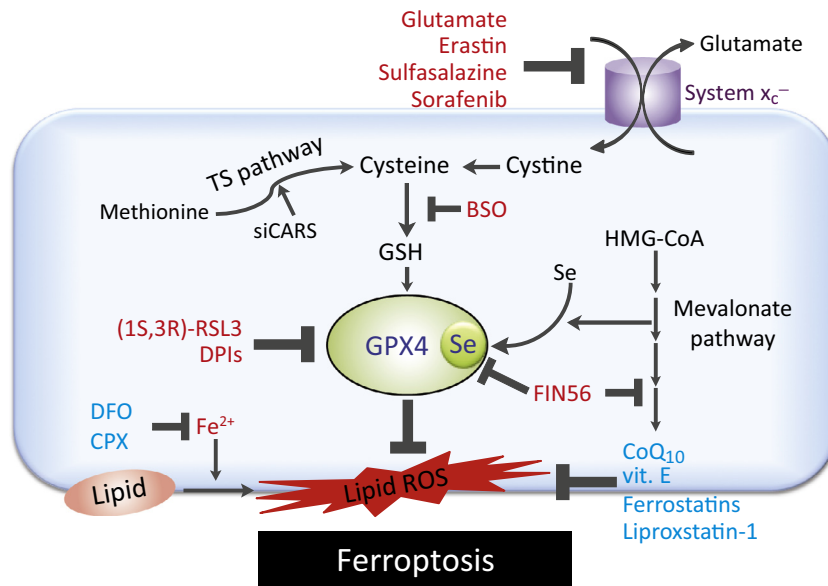
Ferroptosis Induction by System x_c^- Inhibition

Glutamate-induced toxicity can be initiated by calcium influx after glutamate receptor activation [19] or by competitive inhibition of system x_c^- , the glutamate/cystine antiporter [20,21] (Box 1). Glutamate-induced neurotoxicity is an oxidative, iron-dependent process, suggesting that ferroptosis is involved [22,23]. Calcium chelators showed no effect on erastin-induced cell death [14], suggesting that glutamate receptor activation is not involved. In addition, the modulatory profiles of erastin and sulfasalazine (SAS), an inhibitor of system x_c^- , were similar, suggesting that erastin might act as a system x_c^- inhibitor to initiate ferroptosis [10]. Erastin treatment abolished the import of radiolabeled cystine [10], a substrate for the system x_c^- antiporter, confirming that erastin inhibits system x_c^- .

One metabolic consequence of system x_c^- inhibition is depletion of the intracellular cysteine pool, which is a precursor for glutathione synthesis. A metabolite profiling assay revealed that

Key Figure

Molecular Pathways of Ferroptosis Regulation.



Trends in Cell Biology

Figure 1. Small-molecule inducers of ferroptosis are colored red; small-molecule inhibitors of ferroptosis are colored blue. Abbreviations: TS pathway, transsulfuration pathway; Se, selenocysteine; DFO, deferoxamine; CPX, ciclopirox olamine; CoQ₁₀, coenzyme Q₁₀.

glutathione was the most decreased cellular metabolite during erastin-induced ferroptosis [24]. This glutathione depletion is sufficient for erastin-dependent cell death, as glutathione depletion by another reagent, buthionine sulfoxamine (BSO), also initiated ferroptosis [24]. BSO is an inhibitor of glutamate–cysteine ligase, the rate-limiting enzyme for glutathione synthesis. Glutathione depletion causes loss of cellular antioxidant capacity as well as inhibition of glutathione-dependent enzymes such as glutathione peroxidases [24,25].

Direct Inhibition of GPX4 by RSL3

Cell death induced by RSL3 shares common features with erastin-mediated ferroptosis, such as iron, MEK, and ROS dependency [12]. However, RSL3 treatment does not prevent the import of radiolabeled cystine, indicating that RSL3 does not inhibit system x_c^- [10]. Moreover, glutathione is not depleted during RSL3-induced cell death [24], supporting the presence of an alternative ferroptosis-initiating mechanism for RSL3.

Analysis of mass spectrometry-based proteomic data from an affinity pull-down experiment ranked GPX4 (PHGPx) as the top protein target for (1S,3R)-RSL3 [24]; western blotting further confirmed binding between (1S,3R)-RSL3 and GPX4. Cell lysates prepared from (1S,3R)-RSL3-treated cells could not reduce phosphatidylcholine hydroperoxides into the corresponding alcohols, demonstrating that RSL3 inhibits GPX4 enzyme activity [24]. Moreover, knock down of GPX4 expression generates lipid ROS and induces ferroptosis, supporting the hypothesis that (1S,3R)-RSL3 induces ferroptosis through inhibition of GPX4 [24].

Box 1. Cell Death Pathways in Glutamate-Induced Neurotoxicity

When excessive glutamate is present near nerve cells, a nonapoptotic, oxidative form of cell death occurs. As excessive amounts of glutamate inhibit system x_c^- , a cystine/glutamate antiporter, it is likely that glutamate-induced neurotoxicity displays many features of ferroptosis. Nerve cells treated with glutamate show decreased cystine import, become deficient in cellular glutathione, and undergo oxidative cell death that can be prevented by iron chelation [22,88,89]. The term oxytosis has been used to describe this mode of neuronal cell death [22]. Although oxytosis and ferroptosis display related phenotypes, there exist several notable differences. During oxytosis, high levels of calcium ions (Ca^{2+}) enter cells and activate numerous destructive enzymes such as serine proteases, calpains, and phospholipases [90–92]. The elevated calcium ions also damage mitochondria, causing translocation of apoptosis-inducing factor (AIF) from mitochondria to the nucleus [93]. These biochemical changes are required for the execution of oxytosis whereas they are dispensable for ferroptosis. Oxytosis may represent a combination of the more general ferroptosis and a specialized type of damage induced by glutamate through other pathways and receptors.

Normally, glutamate acts a signaling molecule (neurotransmitter) that mediates most excitatory synaptic transmissions in the central nervous system. This glutamate-mediated signaling occurs through the interactions between glutamate and glutamate receptors in nerve cells. It is possible that this glutamate-to-glutamate receptor interaction plays significant roles in oxytosis in nerve cells, whereas inhibition of system x_c^- plays a dominant role in ferroptosis in cancer cells (Figure 1).

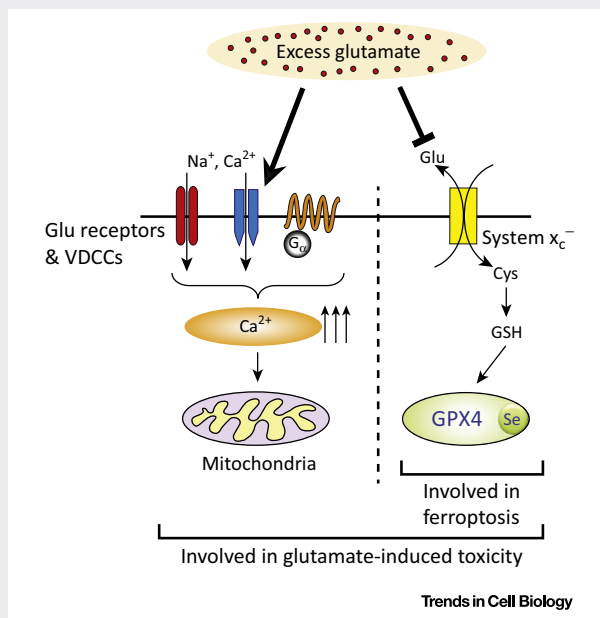


Figure 1. Cell Death Pathways in Glutamate-Induced Neurotoxicity. Abbreviations: VDCCs, voltage-dependent calcium channels.

The identification of GPX4 as the target protein for RSL3 is intriguing for two reasons. First, GPX4 is the only enzyme that can reduce lipid hydroperoxides within biological membranes [25]. This explains why cells treated with RSL3 display elevated lipid ROS during ferroptosis in the absence of GSH depletion. The strong protective effect of lipophilic antioxidants such as vitamin E is likely to be due to their ability to suppress the formation and propagation of oxidized lipids on GPX4 inhibition. Second, GPX4 uses glutathione as an essential cofactor for its enzymatic activity [25]. As erastin inhibits system x_c^- and depletes glutathione, the result is that erastin inhibits GPX4 in an indirect manner, which explains how erastin and RSL3 share a common cell death execution mechanism but have different triggering mechanisms (Figure 1).

Emerging Ferroptosis-Inducing Compounds and Mechanisms

Additional ferroptosis-inducing compounds and mechanisms have been identified and are broadly categorized as system x_c^- inhibitors, glutathione depleters, or direct GPX4 inhibitors.

Induction of Ferroptosis by DPI Compounds

The screening assay in which erastin and RSL3 were identified later revealed ten additional compounds that induce ferroptosis [24,26]. All but one compound (DPI2) inhibited GPX4 directly, similar to RSL3. By contrast, DPI2 depleted GSH, like erastin [24]. Overexpression of GPX4 suppressed cell death induced by these compounds [24] but not other lethal agents, indicating that GPX4 is a specific and robust central regulator of ferroptotic cell death, akin to BCL-2 in apoptosis.

System x_c^- Inhibition by Sorafenib

Sorafenib, a multikinase inhibitor, is an FDA-approved drug used for treating advanced renal cell carcinoma and advanced hepatocellular carcinoma (HCC). In HCC cell lines, sorafenib was reported to induce a nonapoptotic form of cell death that was suppressed by lipophilic antioxidants [27,28], indicating that sorafenib activates ferroptosis in HCC cell lines. Sorafenib acts as an inhibitor of system x_c^- , similar to erastin [29]. The adverse event profile of sorafenib in patients was found to be consistent with the notion that sorafenib exerts additional activity *in vivo* compared with other, similar kinase-inhibiting drugs that do not inhibit system x_c^- [29]. Recently, retinoblastoma protein (Rb) was found to suppress ferroptosis in response to sorafenib [30], suggesting that Rb status may aid in selecting ferroptosis-sensitive tumors, at least for sorafenib.

Ferroptosis Induction by FIN56

CIL56 and FIN56 are small molecules discovered from a systematic survey of nonapoptotic cell death mechanisms [31]. CIL56-induced and FIN56-induced cell death is accompanied by lipid ROS generation; vitamin E and iron chelators suppressed cell death induced by these compounds [31], confirming that they are ferroptosis inducers. Cells treated with FIN56 maintained GSH levels, indicating that, unlike erastin, FIN56 is not a system x_c^- inhibitor. Instead, FIN56 treatment resulted in loss of GPX4 protein through post-translational degradation and blocked mevalonate-derived production of lipophilic antioxidants such as coenzyme Q₁₀ (Figure 1). While FIN56 acts solely as a ferroptosis inducer, CIL56 also activates a nonferroptotic mechanism at higher concentrations and is dependent on ACC1 (acetyl-CoA carboxylase alpha) [32].

Pathways Modulating Ferroptosis

Mevalonate Pathway

Selenoprotein biosynthesis is regulated by the mevalonate pathway. GPX4 is a selenoprotein that contains selenocysteine (Sec) in the active site of the enzyme (Figure 1). The genetic code for Sec is UGA, which is normally a stop codon; therefore, it requires dedicated translational machinery to incorporate Sec into the internal UGA codon of GPX4. The selenocysteine tRNA [tRNA(Sec)] is one of the key components of the machinery and controls the biosynthesis of all 25 selenoproteins present in humans [33]. tRNA(Sec) undergoes lipid modification (isopentenylation) at a specific adenine site during the maturation process, which is required for efficient Sec incorporation into selenoproteins. The enzyme that performs this modification, tRNA isopentenyl transferase, uses isopentenyl pyrophosphate as a donor. As isopentenyl pyrophosphate is a product of the mevalonate pathway, inhibitors of the mevalonate pathway, such as statins, have been shown to interfere with tRNA(Sec) maturation and the biosynthesis of GPX4 in cell culture [34]. Efforts to target the mevalonate pathway in several human diseases, including hypercholesterolemia, osteoporosis, and cancer, suggest that further investigation into the modulation of ferroptosis by the mevalonate pathway is warranted.

The Transsulfuration Pathway

Cysteine is a nonessential amino acid because some mammalian cells are able to synthesize cysteine through the transsulfuration pathway, in which methionine is used as a sulfur donor and is converted into cysteine through the intermediates homocysteine and cystathionine [35]. In

addition to synthesizing cysteine *de novo*, cells can take up extracellular cysteine in the form of cystine, the oxidized disulfide, using transporters such as system x_c^- and excitatory amino acid transporters (EAATs) to provide the cysteine required for protein synthesis and the detoxification of oxidative stress [36].

The relative importance of transsulfuration versus uptake mechanisms is cell-type dependent under normal conditions. The transsulfuration pathway becomes important for cell survival when the uptake mechanism is inhibited. A genome-wide RNAi screen revealed siRNAs targeting cysteinyl tRNA synthetase (*CARS*) as robust genetic suppressors of erastin-induced ferroptosis [37]. Both genes and metabolites in the transsulfuration pathway, such as cystathionine and cysteine, were upregulated in *CARS*-depleted cells treated with erastin [37] (Figure 1). Thus, the transsulfuration pathway is a regulator of ferroptosis; ferroptosis inducers such as erastin that block cystine uptake might be preferentially lethal to cancer cells with a defective transsulfuration pathway.

Additional Pathways Regulating Ferroptosis

Several additional pathways have been reported to modulate ferroptosis. Glutamine and transferrin were found to induce ferroptosis under serum-deprivation conditions, via the glutaminolysis pathway [38,39]. Moreover, inhibiting glutaminolysis was protective in a model of ischemia–reperfusion-induced heart injury, suggesting that this is an additional strategy and indication for suppressing ferroptosis. Acetaminophen has been reported to induce ferroptosis in primary hepatocytes via glutathione depletion [40], suggesting this may be a mechanism of drug-induced toxicity. Finally, heat shock protein beta 1 (HSPB1) was reported to suppress ferroptosis through an unknown mechanism [41]; this suggests that heat shock proteins may be important in setting the sensitivity to ferroptosis in some cell contexts.

Role of Ferroptosis in Development

Recent *in vivo* and clinical studies of components of ferroptosis are beginning to reveal a role for this cell death process in development. Knockout of *Gpx4* in mice caused embryonic lethality between E7.5 and E8.5, indicating an essential role of *Gpx4* in mouse development [42,43]. An inducible *Gpx4* knockout mouse model demonstrated that some cells and tissues, particularly neurons in the brain, accumulate lethal lipid ROS that can be alleviated by vitamin E treatment [44], highlighting the lethal nature of lipid peroxidation *in vivo* in the absence of *Gpx4*. Moreover, a recent study found that conditional ablation of *Gpx4* in neurons in mice caused selective and rapid motor neuron degeneration via ferroptosis, and ultimately onset of paralysis [45]; this suggests that ferroptosis may drive some types of motor neuron disease and its suppression is perhaps critical in motor neuron development and homeostasis. No hallmark of apoptosis could be detected in the dying cells of *Gpx4*^{-/-} mice [44] and analysis of the same animal model confirmed that the lethal phenotype is driven by ferroptosis [15]. Tubular cells in the kidney are also particularly susceptible to ferroptosis induction by *Gpx4* deletion *in vivo* [15], which is intriguing in light of the enhanced sensitivity to erastin of renal cell carcinoma cell lines in culture [24]. In addition, conditional deletion of *Gpx4* in T cells in mice resulted in T cell ferroptosis, resulting in lack of an immune response to infection [46]; this suggests that *Gpx4* is essential for a functional T cell-mediated immune response.

In addition to causing embryonic lethality on loss of *Gpx4*, ferroptosis may be involved in the normal process of mammalian limb development. During development in mammals, interdigital webbing is removed by activation of programmed cell death processes to sculpt the digits [47]. In addition to the presence of apoptotic markers in these interdigital dying tissues, ROS levels are elevated and *Gpx4* expression levels are reduced, suggesting activation of ferroptosis [48]. While the limbs of *Gpx4* transgenic mice do develop normally, possibly arguing against the role of ferroptosis in this process [49], overexpression of *Gpx4* is not likely to overcome

glutathione depletion; moreover, the *Gpx4* transgene may not be activated in this tissue context and suppression of the ferroptosis phenotype *in vivo* may require engineering of multiple ferroptosis regulatory genes. By analogy, the limbs of mice lacking *Bak* develop normally, whereas mice lacking both *Bax* and *Bak* fail to achieve a sufficient level of physiological cell death and display persistent interdigital webbing [50].

Connections to Human Disease

The importance of ferroptosis in development is also evident clinically [51]. Sedaghatian-type spondylometaphyseal dysplasia is a neonatal lethal form of spondylometaphyseal dysplasia, a bone growth disorder, and is inherited in an autosomal recessive manner [52]. Whole-exome sequencing of an affected child and its family identified point mutations in *GPX4* that caused premature truncation of the GPX4 protein, which results in a lack of GPX4 enzyme activity [53]. Numerous other ferroptosis–disease associations are summarized in Table 1.

The role of ferroptosis in various pathophysiological settings has been evaluated using ferrostatin-1 (Fer-1), a small-molecule inhibitor of ferroptosis. Fer-1 acts as a lipid ROS scavenger and prevents ferroptosis induced by erastin and RSL3 but does not suppress cell death induced by staurosporine (an apoptosis inducer) or hydrogen peroxide (an oxidative necrosis inducer) [10].

Acute Kidney Injury

Rhabdomyolysis is the breakdown of muscle tissue due to either direct or indirect muscle injury [54]. Breakdown products, including myoglobin, enter the circulation and damage sensitive tissues such as the kidney, causing acute organ damage such as kidney failure. Iron released from the heme of myoglobin generates ROS and damages kidney cells [55]. Administration of hydroxyquinoline and ferrous ammonium sulfate to freshly isolated mouse kidney proximal

Table 1. Ferroptosis in pathological conditions

Disease	Relevance to ferroptosis
Diffuse large B cell lymphoma (DLBCL)	Intrinsically more sensitive to ferroptosis inducers [24] Dependent on cysteine/cystine import system (system x _c ⁻) due to defective transsulfuration pathway in DLBCL [76,77]
Chromophobe renal cell carcinoma	Intense iron staining in the tumor tissue (iron rich environment) [80]
Acute kidney failure by rhabdomyolysis	Ferrostatin-1 prevented cell death in an ex vivo model of rhabdomyolysis-induced acute kidney injury [57] Myolysis releases myoglobin and heme iron causing ROS generation and acute kidney injury [54]
Acute kidney failure by ischemia-reperfusion injury	16-86, a third generation ferrostatin, protected mice from acute renal failure due to ischemia-reperfusion injury [58]
Huntington's disease	Ferrostatin-1 prevented cell death in an organotypic slice culture model of Huntington's disease [57]
Periventricular Leukomalacia (PVL)	Lipid peroxidation products detected in 33 human autopsy brains with PVL [66] Premature oligodendrocytes (OLs) were more sensitive to GSH depletion-induced cell death [67] Ferrostatin-1 prevented cell death in an OL culture model of PVL [57]
SBP2 deficiency syndrome	Mutation in SBP2 prevents translation of selenoproteins including GPX4 [71] Fibroblasts from patients showed increased basal level of lipid-ROS [71]
Sedaghatian-type spondylometaphyseal dysplasia	Loss of function mutation in GPX4 is associated with neonatal lethality of affected children [53]

tubules simulates major elements of rhabdomyolysis-induced acute kidney injury [56]. Using this model, Fer-1 prevented cell death in kidney proximal tubules, suggesting the necessity of ferroptosis for rhabdomyolysis-induced acute kidney injury [57]. In addition, Fer-1 prevented the synchronized death of renal tubules and oxalate crystal-induced acute kidney injury [58]. SRS16-86 is a third-generation ferrostatin with increased plasma and metabolic stability; in an *in vivo* model of renal ischemia–reperfusion injury, SRS16-86 protected kidney function and extended mouse survival after ischemia–reperfusion injury [58].

Liproxstatin-1, a spiroquinoxalinamine derivative, is able to rescue cell death induced by *Gpx4* knockout [15]. Liproxstatin-1 suppressed cell death induced by ferroptosis inducers (erastin, RSL3, and BSO) but did not rescue cells dying by apoptosis or necroptosis, indicating that liproxstatin-1 is a specific inhibitor of ferroptosis. When liproxstatin-1 was administered to mice with inducible loss of *Gpx4* protein, it delayed the acute renal failure caused by *Gpx4* depletion and extended the survival of mice compared with a vehicle-treated group [15]. These results reinforce the susceptibility of kidney tissue to ferroptosis and suggest the value of ferroptosis inhibitors in ameliorating kidney injury [59].

Huntington Disease

Huntington disease is an inherited neurodegenerative disorder caused by expansion of CAG repeats in the *Huntingtin (HTT)* gene [60]. While the mechanism by which mutant *HTT* induces the disease phenotype is not completely understood, it is known that the toxic effect of mutant *HTT* is functionally associated with glutamate-induced toxicity, glutathione-mediated redox regulation, and cellular iron metabolism [61,62]. Lipid peroxidation products colocalized with mutant *Htt* inclusions in striatal neurons of R6/2 Huntington disease model mice [63]. Intraventricular delivery of deferoxamine, an iron chelator, provided a protective effect in R6/2 mice [64]. When Fer-1 was added to rat corticostriatal brain slices, it prevented neuronal cell death induced by the gene delivery of *HTT* exon 1 fragment with a pathogenic repeat (73Q) [57]. Taken together, the data support the hypothesis that ferroptosis plays a detrimental role in the context of Huntington disease.

Periventricular Leukomalacia (PVL)

PVL is a form of white-matter brain injury that underlies most of the neurological morbidity encountered by many premature infants [65]. At the cellular level, it is caused by loss of oligodendrocytes (OLs), which are particularly vulnerable to neuronal cell death during the trauma of premature birth, contributing to the neuropathogenesis of PVL. Analysis of patient samples demonstrated the presence of abundant lipid oxidation products such as 8-isoprostane and malondialdehyde (MDA) in the cerebrospinal fluid of infants with white matter injuries [66]. Glutathione depletion in rat OL cultures induced cell death that was prevented by vitamin E cotreatment [67]. Fer-1 and its analogs also protected OLs from death by glutathione depletion, suggesting that the ferroptosis pathway is involved in this form of brain injury [57].

Selenocysteine Insertion Sequence Binding Protein 2 (SBP2) Deficiency Syndrome

SBP2 (SECISBP2) binds to a stem–loop RNA structure [Sec insertion sequence (SECIS) element] located in the 3' untranslated region (3'-UTR) region of selenoprotein mRNAs and facilitates the incorporation of tRNA(Sec) into the ribosomal complex at the internal UGA site [68]. Patients with mutations in the *SBP2* gene have been identified who have reduced amounts of most selenoproteins and displayed a multiple-symptom disorder [69–72]. These patients displayed high susceptibility to UV radiation-induced oxidative damage, which is explained by deficiency in the synthesis of glutathione peroxidases. Dermal fibroblasts from the patients generated markedly increased lipid ROS compared with control cells as assessed by BODIPY-C11 staining, suggesting that ferroptosis might also be activated in this context.

Cancer

The possibility of applying ferroptosis inducers to RAS-mutated cancer cells has been investigated by testing erastin in 117 cancer cell lines from diverse tissues [24]. Although erastin displayed selective lethality in engineered cells overexpressing HRAS^{G12V}, there was no consistent difference in erastin sensitivity between wild-type and mutated RAS cells. Relevant to this, ectopic overexpression of oncogenic RAS mutants increased basal ROS levels in fibroblasts through a RAF–MEK–MAPK–NOX pathway, whereas mutations in the endogenous RAS alleles (without overexpression) upregulated NRF2, a transcription factor that induces the expression of antioxidant genes, enhancing the ROS-detoxifying capacity of the cells [73]. The RAS-selective-lethal property of ferroptosis inducers might be masked in RAS-mutated cancer cell lines due to activated NRF2. Consistent with this hypothesis is the report that NRF2 protects against ferroptosis in HCC cells [74].

A recent study reported that p53 can activate ferroptosis by suppressing the expression of SLC7A11, a component of system x_c⁻ [75]. Furthermore, this suppression was suggested to contribute to the tumor suppressive activity of p53, indicating that p53 wild-type tumors may be susceptible to treatment with ferroptosis inducers that inhibit system x_c⁻.

Diffuse large B cell lymphoma (DLBCL) and renal cell carcinomas (RCCs) may be effectively targeted with ferroptosis inducers. DLBCLs are intrinsically more sensitive to ferroptosis than other subtypes of hematologic cancer such as acute myeloid leukemia and multiple myeloma. Since some leukemias and lymphomas have a defective transsulfuration pathway [76,77], which increases their dependence on cysteine/cystine import from the extracellular environment, cysteine/cystine auxotrophy could partially explain the general sensitivity of DLBCL to ferroptosis by system x_c⁻ inhibition. Furthermore, DLBCLs with low system x_c⁻ activity are more susceptible to ferroptosis, as sulfasalazine potently inhibited the growth of lymphomas with low-level expression of SLC7A11, a subunit of system x_c⁻ [77,78]. Furthermore, SLC7A11-deficient mice develop normally and are healthy [79], suggesting that compounds targeting system x_c⁻ with high specificity may have minimal side effects in preclinical and clinical settings.

Moreover, RCC was found to be particularly susceptible to erastin-induced ferroptosis compared with other tissues in the NCI-60 panel, a panel of cancer cell lines derived from eight different tissues [24]. There are three subtypes of RCC based on histological classification: clear cell, papillary, and chromophobe. Chromophobe RCCs can be differentiated from other RCCs based on the intense iron staining in the tumor tissue [80]. In addition, iron consumption by RCC has been considered as the cause of iron deficiency-related anemia in patients [81,82]. In cell culture models, the addition of iron to culture media sensitizes cancer cells to ferroptosis, probably by lowering the threshold to initiate oxidative damage [10]. The iron-enriched tumor environment, as well as the use of sorafenib to treat RCCs in the clinic, suggests that RCCs, especially chromophobe RCCs, could be targeted for ferroptosis induction. Improved erastin analogs such as piperazine erastin (PE) [24] and imidazole ketone erastin (IKE) [83] may be useful as *in vivo* probes of tumor susceptibility to ferroptosis. Of note, artemisinin derivatives have been reported to induce iron-dependent cell death and, specifically, ferroptosis, suggesting they may also be of use in treating such iron-replete tumors [84,85].

Concluding Remarks

An unbiased analysis of cell death pathways has revealed the existence of a previously unappreciated form of regulated cell death: ferroptosis. Several lines of evidence suggest that ferroptosis inducers are not simple oxidizers that, in conjunction with cellular iron, stimulate massive oxidation of biomolecules in the cells, similar to those generated by H₂O₂. First, ferroptosis inducers lack an obvious chemical moiety with redox activity. Second, inhibitors of ferroptosis suppress the lethality of erastin and RSL3 but do not suppress other oxidative

Outstanding Questions

What is the specific role of iron during ferroptosis?

What is the structural basis of GPX4 inhibition by (1S,3R)-RSL3 and related compounds?

What are the specific lipid subclasses that are oxidized during ferroptosis?

What are the molecular downstream pathways after the generation of oxidized lipids during ferroptosis?

What is the best predictive biomarker or signature for ferroptosis sensitivity?

What is the role of ferroptosis in other biological processes?

lethal agents, including H_2O_2 , phenylarsine oxide (a general oxidizer of thiol groups), and rotenone (which generates ROS at mitochondria). If ferroptosis inducers were acting as simple iron-dependent pro-oxidants like H_2O_2 , they would not be specifically suppressed by inhibitors of ferroptosis. The unique nature of GPX4-regulated ferroptosis relative to simple oxidative biocides may be further clarified, as outlined in the Outstanding Questions.

The role of iron remains elusive despite the specific requirement for a cellular iron pool during ferroptosis. Iron acts as a catalyst in converting peroxides into free radicals such as hydroxyl and hydroperoxyl radicals. It was speculated that such Fenton chemistry might account for the lethal effect of ferroptosis inducers. However, the current view favors a more biological route of iron-mediated lipid peroxide generation; copper (Cu^{2+}) and cobalt (Co^{2+}) are known to catalyze Fenton chemistry but these metals do not sensitize cells to ferroptosis, whereas iron does [10]. Moreover, the free radicals generated by Fenton chemistry are highly reactive to biomolecules and induce nonspecific, massive oxidative damage to cells, which is not consistent with the regulated nature of ferroptosis. It is likely that iron-containing enzymes that are associated with lipid redox regulation are activated during ferroptosis. A candidate family of enzymes with this property is the family of lipoxygenases, which catalyze the dioxygenation of polyunsaturated fatty acids in lipids [86]. In addition, the iron-containing heme oxygenase-1 (HO-1) was reported to be induced on erastin treatment and to accelerate erastin-induced ferroptosis [87]; thus, HO-1 may be a source of iron driving the lipid peroxidation that occurs during ferroptosis, at least in response to erastin.

The biological significance of ferroptosis is expanding rapidly by virtue of the discovery that GPX4 and system x_c^- are crucial regulators of ferroptosis as well as the use of ferrostatins to inhibit ferroptosis in diverse contexts. Intriguing questions remain that need to be addressed to improve our understanding of ferroptosis (see Outstanding Questions). Answers to these and other questions will further illuminate the ferroptotic cell death pathway and should be instrumental in translating our knowledge of this basic cell biology to clinical settings.

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