Trends in Molecular Medicine

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Opinion

Targeting Ferroptosis: New Hope for As-Yet-Incurable Diseases

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Attaining control over life and death decisions facilitates the identification of new therapeutic strategies for diseases affected by early cell loss or resistance to cell death. In this context, ferroptosis, a prevailing form of non-apoptotic cell death marked by the iron-dependent oxidative destruction of lipid bilayers and metabolic aberrations, has attracted overwhelming interest among basic researchers and clinicians due to its relevance for a number of degenerative diseases, such as neurodegeneration, ischemia/reperfusion injury (IRI), and organ failure, as well as therapy-resistant tumors. As the ferroptotic death pathway offers various druggable nodes, it is anticipated that the preclinical and clinical development of ferroptosis modulators will unleash unprecedented opportunities for the treatment of as-yet-incurable diseases.

Highlights

Ferroptosis is a pervasive, disease-relevant metabolic cell death pathway, entirely distinct from other known forms of regulated cell death.

Ferroptosis is the root cause of a number of degenerative diseases and emerges as a liability for difficult-to-treat tumors.

Ferroptosis modulation may unleash unprecedented opportunities for pharmacological intervention.

Molecular Principles of Ferroptosis

The traditional perception that cell death can be classified only as apoptosis, the archetypal form of programmed cell death, or **necrosis** (see Glossary), which was long considered to be unregulated with no prospects for pharmacological intervention, is now being challenged by the notion that necrotic cell death can also proceed in a regulated fashion, as first described for **necroptosis** [1]. More than a dozen forms of regulated necrosis have been described, although it remains uncertain whether all of them: (i) are truly independent; (ii) overlap; or (iii) are constrained to certain cell types [2]. Nonetheless, among these forms of regulated necrosis, ferroptosis has sparked tremendous interest as it emerges to be the root cause of a number of degenerative diseases and may provide an Achilles heel for barely treatable tumors [3].

Ferroptosis was first described as a non-apoptotic form of cell death marked by impaired cystine (i.e., the oxidized form of cysteine) uptake into cells, **glutathione (GSH)** depletion and iron-dependent **lipid peroxidation** [4]. However, long before the term ferroptosis was coined, multiple seemingly disparate lines of evidence, obtained by the exploration of various biochemical, cellular, and metabolic processes including cysteine/selenium availability, GSH biosynthesis, iron metabolism, and protection against lipid peroxidation, provided the molecular background for how ferroptosis is currently perceived. Several landmark studies in the first decade of the 21st century on novel cell-death-inducing small molecules and the *in vivo* relevance of the main cellular redox systems in mammals eventually culminated in the recognition of ferroptosis as a distinct form of cell death [4–8]. The discoveries that sufficient cystine supply via the cystine/glutamate antiporter (alias **system x_c^-**) and GSH synthesis are prerequisites for optimal functioning of the selenoenzyme glutathione **peroxidase 4 (GPX4)** and the prevention of lipid peroxidation, established the cyst(e)ine/GSH/GPX4 axis as the key ferroptosis- regulating system in mammals (Figure 1) [9,10].

Genome-wide genetic screens subsequently uncovered a specific fatty acid ligase, **acyl-CoA synthetase long chain family member 4 (ACSL4)**, to determine cells' sensitivity toward

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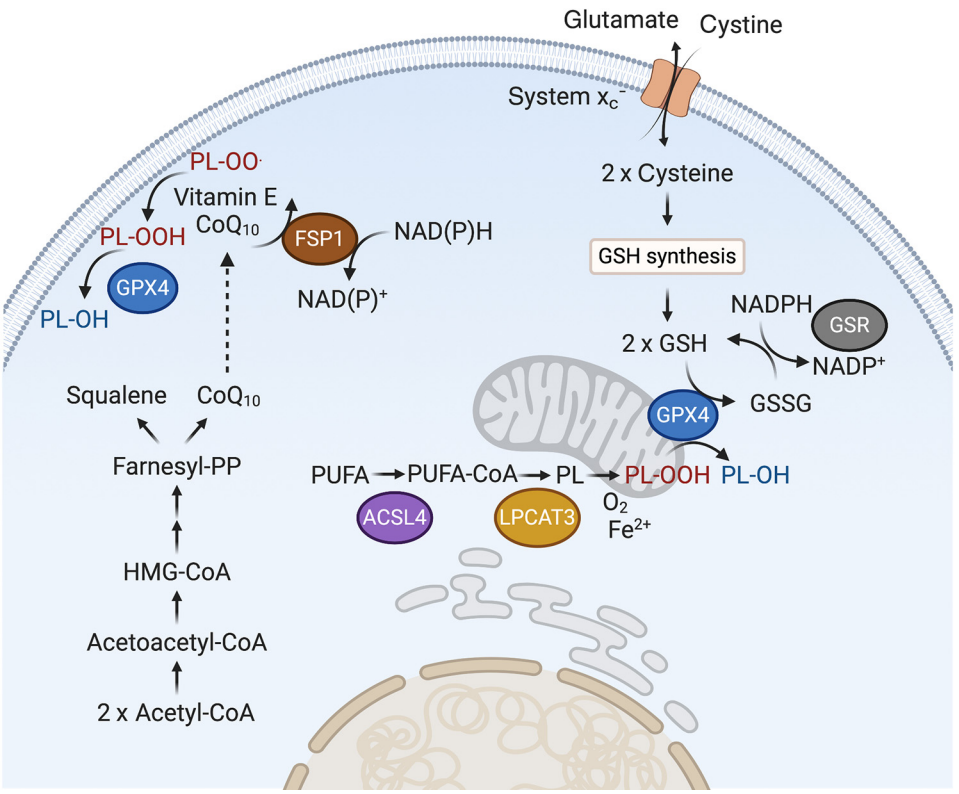

Figure 1. The Ferroptosis Signaling Pathway. Ferroptosis is controlled by two main regulatory systems; namely, the cyst(e)ine/glutathione (GSH)/glutathione peroxidase 4 (GPX4) and the NAD(P)H/ferroptosis suppressor protein 1 (FSP1)/ubiquinone (CoQ_{10}) axis. Ferroptosis prevention via the cyst(e)ine/GSH/GPX4 nexus follows the key steps of cystine uptake and reduction, respectively, GSH biosynthesis, and ultimately the reduction of oxidized phospholipids (PLs) (PL-OOH) to the corresponding alcohols (PL-OH) by GPX4, using GSH as a substrate. Acyl-CoA synthetase long chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) are directly involved in the incorporation of polyunsaturated fatty acids (PUFAs) into cellular membranes, rendering them sensitive toward ferroptosis induction. Thereby, the oxidation of lipid bilayers might occur enzymatically and/or nonenzymatically via auto-oxidation. In the NAD(P)H/FSP1/CoQ_{10} system, the antiferroptotic role of FSP1 is owed to its oxidoreductase activity by reducing extramitochondrial CoQ_{10} to ubiquinol using $NAD(P)H/H^+$. Ubiquinol prevents lipid peroxidation, either directly by reducing lipid radicals (PL-OO·) or indirectly using vitamin E (α-tocopherol). In addition, the mevalonate pathway provides precursors for the biosynthesis of CoQ_{10} and squalene; the latter has been shown to contribute to ferroptosis suppression through its antioxidant activity. Abbreviations: GDR, glutathione-disulfide reductase; HMG-CoA, β-hydroxy β-methylglutaryl-CoA.

ferroptosis [11,12]. The role of ACSL4 in ferroptosis is based on its activity to activate long chain **polyunsaturated fatty acids (PUFAs)** , which, when incorporated into phospholipids, may increase the risk of lipid peroxidation [12,13]. Although lipid peroxidation and the associated rupture of cellular membranes is the hallmark of ferroptosis, it is still intensely debated whether this process is mediated by certain lipoxygenases or by the iron-dependent, radical-mediated **Fenton reaction** and autoxidation of lipid bilayers [7,10,14–17]. Either way, inhibitors of lipid peroxidation or strategies to lower the abundance of PUFAs in membranes are the most efficient means to prevent ferroptosis. Likewise, chelation of iron was repeatedly reported to protect against lipid peroxidation and ferroptotic cell death [4,10], albeit that the precise role of iron in ferroptosis clearly deserves further investigation. Potential intracellular sources of labile redox-active iron include autophagic degradation of the iron-storage protein ferritin [18,19], heme oxygenase-1-mediated heme degradation [20,21], and during an iron-starvation response [22].

Glossary

Acyl-CoA synthetase long chain family member 4 (ACSL4): one of five closely related fatty acid ligases that preferably activates long PUFAs by ligating them with coenzyme A.
Chloroacetamide: a reactive group on small molecules that can covalently inactivate the active-site selenocysteine of selenoproteins.
Fenton reaction: describes a chemical reaction between ferrous iron (Fe^{2+}) and hydrogen peroxide leading to the formation of the highly toxic hydroxyl radical ($OH^·$), which can initiate lipid peroxidation and ferroptosis.
Ferroptosis suppressor protein 1 (FSP1): the second mainstay in ferroptosis control, which acts independently of the GSH/GPX4 axis by regenerating CoQ_{10}.
Glutathione (GSH): a tripeptide comprising glutamate, cysteine, and glycine representing the most abundant intracellular electron donor for GSH-dependent enzymes in mammals (up to 10 mM).
Glutathione peroxidase 4 (GPX4): one of eight GPXs in mammals with unique enzyme activity to efficiently reduce lipid peroxides in cellular membranes thereby preventing lipid peroxidation.
Ischemia/reperfusion injury (IRI): a disease condition whereby the oxygen supply to parts of an organ is transiently disrupted by occlusion of the vessel by a blood clot or during surgery. Restoration of the blood flow (i.e., reperfusion) may cause oxidative stress, massive cell death, and tissue injury.
Lipid peroxidation: oxidative degradation of lipids, whereby free radicals 'steal' electrons from the lipids (i.e., PUFAs) in cellular membranes. This causes the formation of lipid radicals, which can spark the lipid peroxidation chain reaction. If not counteracted by antioxidant enzymes or RTAs, membranes are oxidatively destroyed leading to the rupturing of cells and ferroptosis.
Necroptosis: the first described form of regulated necrosis uncovered by the recognition that tumor necrosis factor alpha (TNFα) induces not only apoptosis and inflammation, but also necroptosis under specific cell contexts.
Necrosis: unregulated forms of cell death mostly induced by physical impacts and chemical toxins that cannot be prevented by cell-death inhibitors.

In search of an as-yet-unrecognized ferroptosis resistance mechanisms, two groups independently discovered **ferroptosis suppressor protein-1 (FSP1)** [previously called 'apoptosis inducing factor mitochondria associated 2' (AIFM2)] as a novel ferroptosis regulator. FSP1 proved to be a highly powerful antiferroptotic enzyme whose overexpression led to complete rescue from ferroptosis induced by genetic deletion or pharmacological inhibition of GPX4 [23,24]. The antiferroptotic function of FSP1 is based on its oxidoreductase activity to reduce extramitochondrial **ubiquinone (CoQ_{10})** to ubiquinol using NAD(P)H/H^+ [23]. Ubiquinol in turn prevents the lipid peroxidation chain reaction, either directly by reducing lipid radicals or indirectly via α-tocopherol, the prevailing form of vitamin E. Thus, the NAD(P)H/FSP1/CoQ_{10} axis acts independent of the canonical GPX4-dependent nexus and does not require selenium for proper functioning [25].

Besides these two main ferroptosis-suppressing systems, squalene, an intermediate metabolite of the cholesterol pathway, as well as GTP cyclohydrolase-1 and its metabolic products di/tetrahydrobiopterin have been linked to ferroptosis resistance by acting as antioxidants, thus preventing uncontrolled lipid peroxidation [26–28].

Conceptual Mechanisms of Ferroptosis in Health and Disease

In recent years, ferroptosis has been linked with various pathological conditions, ranging from degenerative diseases like **ischemia/reperfusion injury (IRI)**, organ failure, and neurodegeneration to certain therapy-resistant cancer entities and metastasis formation [3]. Most of these studies on the tissue-protective role of ferroptosis suppression originated from studies using transgenic mice (Box 1 and Figure 2), and the use of ferroptosis-inhibiting compounds. While the physiological implications of ferroptosis are still being unraveled, it is believed that protection from ferroptosis is an evolutionary requirement, which arose from the incorporation of PUFAs into cellular membranes to support the formation of complex organisms, neuronal networks, and mammalian development [29]. Tumor cells, by contrast, exploit PUFA-enriched environments and associated changes in cellular plasticity to switch to a therapy-resistant state or to undergo epithelial–mesenchymal transition, which represents an attractive vulnerability for therapeutic intervention [25]. To date, compelling evidence exists for the relevance of ferroptosis in animal models of disease, although proof-of-concept studies in patients remain in their infancy.

Polyunsaturated fatty acids (PUFAs): fatty acids containing more than one double bond in their backbone.
Radical-trapping antioxidants (RTAs): type of small molecule-compounds including alpha-tocopherol, liproxstatin, and ferrostatin that can stop the process of lipid peroxidation at the level of lipid radicals.
Selenocysteine: the 21st proteinogenic amino acid, differing from its analog cysteine only by the replacement of sulfur with selenium.
Selenoprotein: a small family of 25 dedicated proteins in humans that contain the 21st, rare amino acid selenocysteine.
System x_C^-: a heterodimeric cystine/glutamate amino acid transporter comprising xCT (SLC7A11) and 4F2 (SLC3A2), importing cystine in cells while releasing one molecule of glutamate.
Ubiquinone (CoQ_{10}): also known as coenzyme Q_{10}; a lipophilic metabolite derived from the mevalonate pathway that usually functions in the mitochondrial electron transport chain. Extramitochondrial CoQ_{10} regenerated by FSP1 acts like a strong RTA and regenerates vitamin E to prevent lipid peroxidation.

Box 1. Tissue-Specific Susceptibilities toward Ferroptosis in Mice

Since the discovery of ferroptosis as a distinctive form of necrotic cell death, there has been an extensive search for both the molecular and the metabolic determinants that determine cells' sensitivity toward ferroptosis. While it is still far from being understood why certain tissues or even cells in a given tissue are susceptible to ferroptosis, transgenic mouse studies have been instrumental in delineating ferroptosis-sensitive versus -resistant tissues (see Figure 2 in main text). Since GPX4 is crucial for early embryonic development [75], tissue-specific *Gpx4*-knockout studies unveiled that certain neuronal subpopulations, including hippocampal neurons [7], glutamatergic neurons and parvalbumin-positive interneurons of the cortex [29,76], cerebellar Purkinje cells [77], and motoneurons [46], are strictly dependent on functional GPX4, while certain neuronal subpopulations of the hypothalamus and dopaminergic neurons are resistant to GPX4 loss [78]. Outside the brain, proximal kidney tubular cells are the most ferroptosis-sensitive cell type, since the inducible *Gpx4* knockout causes acute kidney injury resembling that of delayed graft function as occurs intermittently during organ transplantation [10]. Also, hemostasis of CD8-positive T cells, reticulocyte maturation, and innate-like B1 and marginal zone B cells require GPX4 expression [65,79,80]. Notably, in certain tissues *Gpx4* deficiency can be bypassed by dietary vitamin E levels, which can be remarkably high in most chows used for mouse experiments. For instance, endothelium-specific *Gpx4*-knockout mice kept under regular dietary conditions are phenotypically normal, while under low vitamin E endothelial cell death causes thrombosis, multiple microinfarcts, and death in mice [81]. A similar finding has been reported for hepatocyte-specific GPX4-null mice [82]. Hence, these findings warrant careful planning when interrogating the *in vivo* relevance of ferroptosis not only in (patho)physiological contexts, but also when assessing the *in vivo* pharmacological potential of novel ferroptosis modulators.

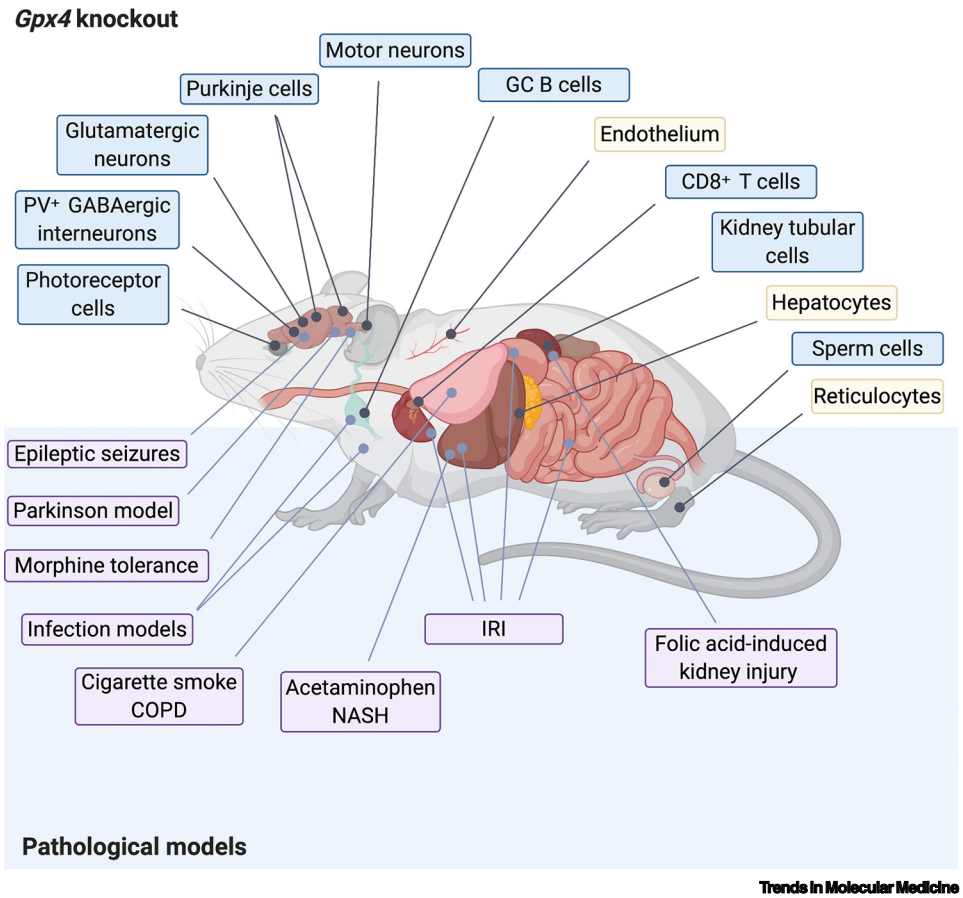

Figure 2. Tissues, Cells, and Pathological Conditions Relevant to Ferroptosis *In Vivo*. Transgenic studies of glutathione peroxidase 4 (GPX4) in mice show which tissues and cells depend on functional GPX4 and are prone to undergo ferroptosis (blue and yellow boxes). In tissues/cell types depicted in yellow boxes, loss of GPX4 can be compensated by vitamin E supplementation (upper panel). Ferroptosis seems to be a root cause of several degenerative diseases and ferroptosis inhibitors show protective effects in a number of pathological disease models in mice (lower panel). Abbreviations: COPD, chronic obstructive pulmonary disease; GC, germinal center; IRI, ischemia/reperfusion injury; NASH, nonalcoholic steatohepatitis; PV, parvalbumin.

Ferroptosis as the Key Driver of IRI and Organ Failure

IRI underlies several disorders, including stroke, cardiac infarction, and other conditions where the blood flow into the organ is interrupted for a given period of time (e.g., during surgical intervention or organ transplantation) [3]. In this context, ferroptosis inhibitors have been shown to successfully protect against IRI in various organs including the liver [10], kidney [30], intestine [31], and lung [32], during myocardial infarction [33,34], and in stroke [35] (Figure 2). Additional tissue-protective effects of ferroptosis inhibitors have been reported in models of acute renal failure [10], intracerebral hemorrhage [36], nonalcoholic steatohepatitis (NASH) [37–39], and epileptic seizures [40]. Beyond this, several features of ferroptotic cell death have been found in models of traumatic brain injury [41], hemochromatosis [42], and acetaminophen-induced acute liver failure [43] and urine samples from patients with acute kidney injury [41]. It has been further proposed that ferroptosis occurs as prime event on the initial insult of tissue injury, followed by secondary activation of alternative regulated necrotic cell death pathways and necroinflammation [44]. Therefore, patients with acute diseases such as cardiac infarction or stroke would benefit most from immediate intervention therapies with ferroptosis inhibitors. In

the special case of organ transplantation, preventive *ex vivo* application by flushing of the donor organ prior to implantation is desirable to avoid IRI and subsequent secondary complications.

Possible Role of Ferroptosis in Neurodegenerative Disease

The early and progressive loss of neuronal cells is a common feature of neurodegenerative diseases (NDs). Ferroptotic cell death has been implicated in brain regions affected by Alzheimer's disease and dementia [7,45] as well as amyotrophic lateral sclerosis (ALS) [46]. Moreover, ferroptosis inhibitors ameliorated the toxic effects in the MPTP Parkinson model [47] and inhibited neuronal demise in an *ex vivo* model of Huntington's disease [48]. Edaravone, an approved drug for ALS treatment, prevents ferroptosis *in vitro*, albeit only at relatively high concentrations [49]. Therefore, it remains to be formally shown whether brain/spinal cord concentrations of edaravone in patients reach sufficient levels to warrant ferroptosis inhibition *in vivo*. Deuterated-PUFAs (D-PUFAs) are another class of drug candidates with ferroptosis-inhibiting activity. D-PUFA supplementation facilitates gradual remodeling of cellular membranes and therefore prevents uncontrolled lipid peroxidation and associated ferroptosis [14]. At present, clinical trials are ongoing or planned in patients with various neurodegenerative conditions known to involve lipid peroxidation, like Friedreich's ataxia, infantile neuroaxonal dystrophy, ALS, progressive supranuclear palsy, Huntington's disease, GM1 gangliosidosis, and late-onset Tay–Sachs disease [50]. Recently, Cu-ATSM, a new investigational drug that improves neurodegeneration and delays disease progression in mouse models of ALS and Parkinson's disease, has been reported to possess antiferroptotic properties [51]. The currently ongoing clinical trials in NDs using Cu-ATSM and D-PUFAs thus hold great promise to prove whether ferroptosis is a relevant pathway for pharmacological intervention in patients.

Ferroptosis Susceptibility as a Promising Vulnerability for Cancer Treatment

Beyond multiple degenerative disease scenarios, it has become evident that targeting ferroptosis presents a unique vulnerability for the treatment of certain therapy-resistant tumors. Despite the development of targeted therapies, therapy evasion and the development of resistance and metastasis still pose major problems [52]. Even with very effective tumor therapies that lead to partial or complete response, small populations of cancer cells frequently survive drug treatment. The establishment of a drug-tolerant state is often accompanied by a phenotypic switch and change in plasticity. Thereby, tumor cells primarily incorporate PUFAs into their membranes and thus acquire a high dependency on GPX4, associated with increased ferroptosis sensitivity [53–55]. Triple-negative breast cancer (TNBC) is a difficult-to-treat tumor entity that is frequently refractory to standard chemotherapy regimens. However, TNBC cell lines express ACSL4 and preferentially incorporate PUFAs into their cell membranes, which in turn renders them sensitive to ferroptosis [12]. Besides inhibiting GPX4, targeting system x_c^- may be at first glance a viable approach to halt tumor growth. Unlike *Gpx4*, knockout of *Slc7a11* is well tolerated in mice [56], precluding systemic side effects. Several studies already showed that the targeted knockout of *SLC7A11* is sufficient to abrogate tumor growth in various mouse models [57–59]. However, one needs to bear in mind that: (i) system x_c^- activity may be bypassed by other metabolic pathways; (ii) redox conditions vary greatly between cell culture and a whole organism; and (iii) system x_c^- is only selectively expressed in certain cancers [23].

With the recent discovery that the FSP1/CoQ_{10} axis is a powerful system to complement loss of GPX4 [23,24], inhibitors against this system might be important to combat tumors that are resistant to compounds targeting the thiol-dependent ferroptosis-controlling system. Ferroptosis-based therapies targeting the cyst(e)ine/GSH/GPX4, the FSP1/CoQ_{10} axis, or a combination thereof would thus have great potential for many resistance mechanisms arising from cellular plasticity switches, preventing therapy evasion and metastasis in malignancies from various origins.

Small-Molecule Compounds and Other Approaches for the Modulation of Ferroptosis

As for any other type of cell death, the inhibition or induction of ferroptosis holds great promise for the treatment of diseases marked by early cell loss or uncontrolled proliferation. This is especially true for ferroptosis because it: (i) entails a number of metabolic networks possibly involving multiple targets; (ii) is directly controlled by several enzymatic systems; and (iii) involves oxidative modifications of phospholipids with still-untapped pharmacological potential. Therefore, the most widely used ferroptosis modulators are summarized below (Table 1) and their potential to be developed as new medications is discussed in the following sections.

Ferroptosis Inhibitors

Ferrostatin-1 and liproxstatin-1 are the two archetypal ferroptosis inhibitors that have been widely used in various cellular and pathological contexts since their initial discovery [4,10]. As ferrostatin-1 is metabolically unstable and unsuitable for *in vivo* use, improved versions applicable for *in vivo* administration have been developed circumventing this limitation [30,60,61]. Nonetheless, both types of scaffold prevent ferroptosis at its core mechanism; namely, by acting as so-called **radical-trapping antioxidants (RTAs)** [62]. Similar to the lipophilic, naturally occurring antioxidant vitamin E [4,7], they stop the lipid peroxidation chain reaction at the level of lipid radicals by donating one electron. Unlike vitamin E, which is consumed during this process, requiring its recycling via ascorbate or FSP1/CoQ_{10}, these RTAs form nitroxides at a critical amine residue allowing them to function in a catalytic manner [62]. This mechanism of action

Table 1. Summary of the Most Widely Used Ferroptosis Modulators

Name	Disease	*In vivo* use	Preclinical development	Clinical testing	Refs
Inhibitors					
Ferrostatin-1		No	No	No	[4]
Improved ferrostatins	Unknown	Yes	Unknown	No	[30,60,61]
Liproxstatin-1		Yes	No	No	[10]
Improved liproxstatins	IRI, ND	Yes	Yes	No	Personal communication
Edaravone	ALS	Yes	Yes	Approved	[49]
D-PUFAs (RT001)	Friedreich's ataxia and other ND	Yes	Yes	NCT03570931[a], NCT04102501[a]	[50]
Cu-ATSM	ALS and other ND	Yes	Yes	NCT04082832[a], NCT04313166[a]	[51]
Inducers					
Erastin	Cancer	No	No	No	[5]
IKE	Cancer	Yes	Unknown	No	[83]
RSL3	Cancer	No	No	No	[6]
ML162	Cancer	No	No	No	[9]
ML210	Cancer	Unknown	Unknown	No	[84]
JKE-1674	Cancer	Unknown	Unknown	No	[68]
Cyst(e)inase	Cancer	Yes	Unknown	No	[71]
FINO2	Cancer	Unknown	No	No	[85]
iFSP1	Cancer	Unknown	No	No	[23]

[a]These studies are registered with ClinicalTrials.gov.

seems to be shared among several more recently described ferroptosis inhibitors like phenothiazines and phenoxazines, Cu-ATSM, edaravone, and even some previously considered 'lipoxygenase-specific' inhibitors [16,49,51,63,64]. For the latter, this is likely to be the reason why there is still intense discussion about whether lipoxygenases contribute to ferroptosis or just merely have a bystander effect, as clear genetic evidence is widely lacking [10,65]. Either way, natural compounds such as flavonoids, vitamin E, and these small-molecule compounds act as RTAs and thus lack a classical protein target, but instead prevent ferroptosis at the level of lipid peroxidation. An alternative approach involves the use of D-PUFAs, which, when incorporated into lipid bilayers, are quite resistant to peroxidation (due to the so-called isotope effect), thereby acting as antiferroptotic agents by suppressing uncontrolled lipid autoxidation [14,16]. Efforts are under way to provide clinical proof of concept for D-PUFAs, CoQ_{10} analogs, Cu-ATSM, and improved next-generation ferrostatins and liproxstatins.

In accordance with the role of iron in ferroptosis, iron chelators have been frequently used to halt the Fenton reaction and associated lipid peroxidation and have been shown to ameliorate certain pathological contexts [33,38,66]. Future investigations are warranted to assess the therapeutic efficacy of iron chelators in ferroptosis-relevant (neuro)degenerative diseases and those linked to iron overload.

Unlike these 'untargeted' approaches, the discovery that expression of ACSL4 determines sensitivity toward ferroptosis, at least in some cellular contexts [12], and that knockout of ACSL4 or its inhibition with thiazolidinediones, such as rosiglitazone and pioglitazone, markedly protects against ferroptosis, may spark future efforts to test this class of inhibitors in ferroptosis-related diseases [12]. Since thiazolidinediones were originally developed as peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists and insulin sensitizers, it would be certainly worth revaluating earlier studies using rosiglitazone under degenerative disease conditions.

Ferroptosis Inducers

The recognition that therapy-resistant cancers and cancer cells of the mesenchymal state display high vulnerability toward ferroptosis may offer unprecedented opportunities for the development of *in vivo*-applicable ferroptosis-inducing agents (FINs) – both targeted and untargeted – to eradicate difficult-to-treat cancers [53–55]. Meanwhile, an impressive number of FINs have been described, targeting various nodes of the ferroptotic death pathway. Above all are the GPX4 inhibitors RSL3 and ML162 [9], which, however, both contain a highly reactive **chloroacetamide** group and covalently inactivate the active site **selenocysteine** not only of GPX4 but also of most other **selenoproteins** [67], thus limiting their *in vivo* use. This might be bypassed by the discovery that ML210 and related masked nitrile-oxide electrophiles, which also target GPX4, act as prodrugs with clearly improved specificity [68]. Besides these, the natural product withaferin A, a steroidal lactone traditionally used in ayurvedic medicine, targets GPX4 in a dose-dependent manner and efficiently combats high-risk neuroblastoma [21]. Nonetheless, in any case it remains to be shown that targeting GPX4 allows a sufficient therapeutic window due to GPX4's known essentiality for various tissues and organs [3] (Box 1 and Figure 2).

Erastin is a highly specific and irreversible system x_c^- inhibitor [4,69]. Initial limitations due to metabolic instability and solubility have been partly overcome by the synthesis of improved versions like imidazole ketone erastin [70]. Another way to interfere with cyst(e)ine availability is the use of cyst(e)inase, a genetically engineered glutamate–cysteine ligase that degrades cyst(e)ine in mice and nonhuman primates, thereby restraining tumor growth in several tumor models in mice [71]. In addition, treatment with cyst(e)inase in combination with checkpoint inhibitors has shown beneficial effects for cancer immunotherapy [72].

The first-described FSP1 inhibitor, iFSP1, is able to synergize with RSL3 to increase tumor cells' responsiveness to ferroptosis across a wide panel of tumor cell lines [23]. However, future studies are needed to demonstrate whether inhibition of FSP1 alone is sufficient to abrogate tumor growth or whether combination therapies are required to induce efficient tumor cell death. Besides these targeted approaches, the delivery of ferroptotic nanoparticles with toxic cargoes, such as redox-active iron and/or PUFAs, may represent a promising way either as a standalone or combinatorial strategy to treat ferroptosis-responsive tumors [73,74].

Concluding Remarks

Numerous reports ranging from fundamental mechanistic discoveries to state-of-the-art preclinical animal models have been published over the past years to pinpoint the underlying molecular and metabolic determinants and to emphasize the importance of ferroptosis in various disease scenarios. In addition, unequivocal evidence for the pharmacological tractability of ferroptosis has been provided by the discovery of small-molecule ferroptosis modulators, which have the potential to eventually evolve into successful drug candidates. The translation of these exiting concepts for patient benefit is imminent and the first clinical trials have just begun (see Outstanding Questions). However, to unequivocally monitor the therapeutic efficacy of future ferroptosis-targeting drug candidates, new ferroptosis-specific pharmacodynamic markers or biomarkers are urgently needed and await discovery.

Outstanding Questions

Will ferroptosis-inhibiting drugs afford sufficient protection against acute and chronic diseases, such as neurodegeneration, ischemic organ injuries, and acute organ failure?

Will future ferroptosis-inducing drugs in the context of cancer allow an adequate therapeutic window?

Will it be possible to develop biomarkers and pharmacodynamic markers to unambiguously demonstrate ferroptosis engagement in affected patients?

Is there a contribution of ferroptosis in physiology comparable with that of apoptosis or is it related only to pathophysiological conditions?

Will ferroptosis inhibitors cause potential side effects by influencing the as-yet-unknown physiological functions of ferroptosis, especially in the context of the treatment of chronic conditions, such as neurodegeneration?

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