Ferroptosis: mechanisms, biology and role in disease

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Abstract | The research field of ferroptosis has seen exponential growth over the past few years, since the term was coined in 2012. This unique modality of cell death, driven by iron-dependent phospholipid peroxidation, is regulated by multiple cellular metabolic pathways, including redox homeostasis, iron handling, mitochondrial activity and metabolism of amino acids, lipids and sugars, in addition to various signalling pathways relevant to disease. Numerous organ injuries and degenerative pathologies are driven by ferroptosis. Intriguingly, therapy-resistant cancer cells, particularly those in the mesenchymal state and prone to metastasis, are exquisitely vulnerable to ferroptosis. As such, pharmacological modulation of ferroptosis, via both its induction and its inhibition, holds great potential for the treatment of drug-resistant cancers, ischaemic organ injuries and other degenerative diseases linked to extensive lipid peroxidation. In this Review, we provide a critical analysis of the current molecular mechanisms and regulatory networks of ferroptosis, the potential physiological functions of ferroptosis in tumour suppression and immune surveillance, and its pathological roles, together with a potential for therapeutic targeting. Importantly, as in all rapidly evolving research areas, challenges exist due to misconceptions and inappropriate experimental methods. This Review also aims to address these issues and to provide practical guidelines for enhancing reproducibility and reliability in studies of ferroptosis. Finally, we discuss important concepts and pressing questions that should be the focus of future ferroptosis research.

Cells represent the fundamental organizing unit of life. Cell proliferation, differentiation, and functional characteristics, and, ultimately, cell death are therefore of critical importance in the myriad manifestations of life. The fate and functions of cells are impacted by both environmental and genetic cues. One of the most critical axes for cell fate determination is how cells respond to oxidative stress, as most living organisms rely on oxygen as the ultimate electron acceptor in reductive/oxidative (redox)-based metabolic processes. Among the factors that cause oxidative stress in cells, oxidative modification of lipids in membrane bilayers — in particular, lipid peroxidation — has emerged as an important regulator of cell fate, with extensive lipid peroxidation committing cells to death via a distinct cell death paradigm termed ferroptosis. Ferroptosis is now appreciated as likely one of the most widespread and ancient forms of cell death: although originally studied in mammalian systems[1], ferroptosis-like cell death has also been observed in evolutionarily remote species, such as those belonging to the kingdoms of plants, protozoa and fungi[2–4]. Notably, lipid peroxidation integrates a series of environmental and genetic inputs, including heat and radiation exposure, metabolism, redox homeostasis

and intercellular contacts, as well as oncogenic and tumour-suppressive signalling. In line with these inputs into ferroptosis induction, mounting evidence suggests potential physiological roles of ferroptosis in tumour suppression and immunity (FIG. 1). More recently, ferroptosis has also been implicated in the normal development of certain fungal species[4] and in developmental ageing of nematodes[5]. Furthermore, the pathophysiological relevance of ferroptosis, especially as a therapeutic modality in cancer treatment and in preventing ischaemic organ damage, has been convincingly established (FIG. 1).

Although the term ferroptosis was coined fairly recently, in 2012, aspects of, or specialized forms of, ferroptosis-like cell death were observed long before — for instance, a type of oxidative-stress-induced cell death in neuronal cells termed 'oxytosis'[6] and in metabolism studies in the mid-twentieth century. Pioneering work on what we now appreciate as ferroptosis-like cell death performed by Harry Eagle in the 1950s and 1960s showed that deprivation of the amino acid cysteine can cause cell death[7] and that the endogenous synthesis of cysteine makes cells resistant to such cell death[8,9]. Now, we appreciate that cysteine is rate-limiting for the biosynthesis of

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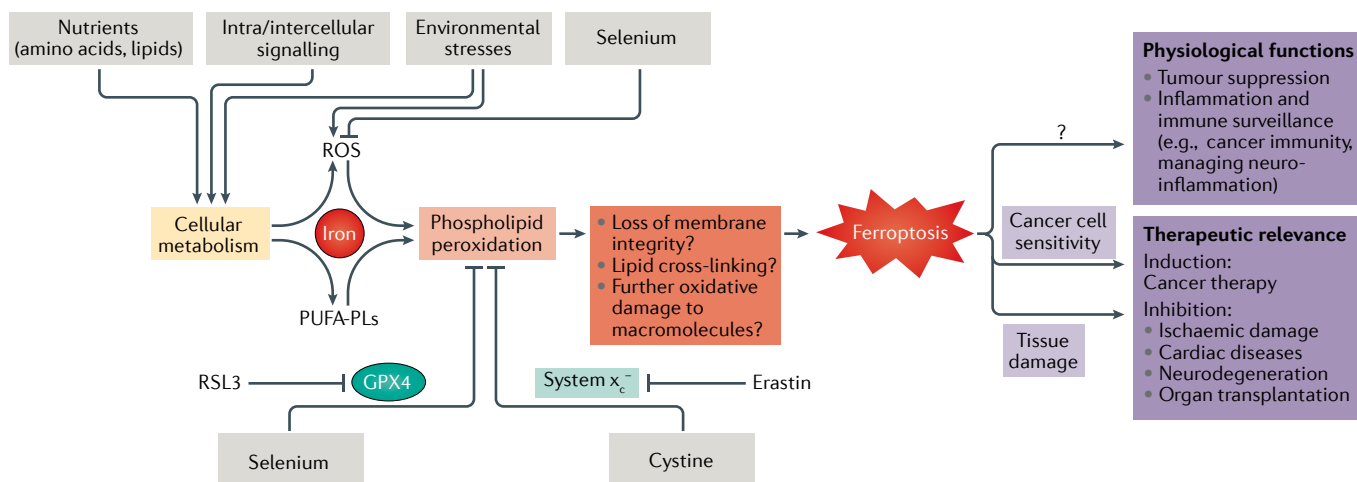✉e-mail: jiangx@mskcc.org; bstockwell@columbia.edu; marcus.conrad@helmholtz-muenchen.de

Fig. 1 | **An overview of ferroptosis.** Cell death via ferroptosis is executed by phospholipid peroxidation, a process relying on the transition metal iron, reactive oxygen species (ROS) and phospholipids containing polyunsaturated fatty acid chains (PUFA-PLs). In addition, cell metabolism can influence levels of both ROS and PUFAs. Various extracellular factors also contribute to ferroptosis susceptibility. An important regulator of ferroptosis is the micronutrient selenium, which is required for the biosynthesis of ROS-scavenging selenoproteins, including a key inhibitor of phospholipid peroxidation, gluta-thione peroxidase 4 (GPX4). Cystine (the oxidized form of cysteine) — after uptake by the system x_c^- cystine/glutamate antiporter — also opposes ferroptosis by contributing to GPX activity (see FIG. 2). In addition, intracellular and intercellular signalling events and environmental stresses can impact ferroptosis by regulating cellular metabolism and ROS levels. Furthermore, the cell metabolic state is regulated by nutrient supply. Ferroptosis has been linked to various pathologies associated with tissue damage, whereby ferroptotic cell death contributes to cell loss. Hence, inhibition of ferroptosis could be a useful therapeutic strategy for these conditions. Curiously, many cancer cells show increased susceptibility to ferroptosis, and ferroptosis induction could be explored as an anticancer therapy. Ferroptosis may also have physiological functions in tumour suppression and immune surveillance, although further studies are required to unambiguously validate these functions (indicated with a question mark). It also should be noted that the exact mechanism responsible for the eventual execution of ferroptotic cell death is elusive, and could include loss of membrane integrity, perturbation of membrane properties via lipid cross-linking and further oxidative damage to macromolecules and cellular structures triggered by PUFA-PL-derived ROS.

Lipid peroxidation
The oxidative destruction of lipids, whereby free radicals snitch electrons from polyunsaturated fatty acid residues in cellular membranes. This in turn leads to the formation of carbon-centred lipid radicals, which can initiate the lipid peroxidation chain reaction if not inhibited enzymatically or by lipophilic antioxidants.

Iron–sulfur clusters
Small molecular structures consisting of iron and sulfur, which are mostly integrated into enzymes and larger protein complexes mediating electron transfer.

Glutathione peroxidases
(GPXs). A family of structurally related enzymes that usually reduce hydrogen peroxide (H_2O_2) and other organic peroxides to water and alcohols, respectively, using mostly glutathione (GSH) as an electron donor.

Glutathione-S-transferases
A large family of enzymes that catalyse the conjugation of the reduced form of glutathione (GSH) to xenobiotics (via a sulfhydryl group) for detoxification purposes.

Thiol deprivation
A cellular condition marked by cysteine deprivation, causing glutathione (GSH) depletion and endoplasmic reticulum stress.

Reactive oxygen species
(ROS). Partially reduced forms of oxygen, such as hydroperoxides, superoxide, singlet oxygen and hydroxyl radicals, that may oxidize and thereby destroy cellular components including lipids, protein and DNA.

reduced glutathione (GSH)[10]. GSH, the most abundant reductant in mammalian cells, is important for the biogenesis of iron–sulfur clusters and is a cofactor for multiple enzymes, including glutathione peroxidases (GPXs) and glutathione-S-transferases. For GSH synthesis, cysteine can be either taken up from the environment — by a neutral amino acid transporter or in its oxidized form (cystine) by the system x_c^- cystine/glutamate antiporter (a transmembrane protein complex containing subunits SLC7A11 and SLC3A2; referred to as system x_c^- throughout the manuscript)[11,12] — or synthesized using methionine and glucose in the trans-sulfuration pathway. It has been demonstrated genetically that sustaining GSH synthesis or promoting the activity of system x_c^- or that of glutathione peroxidase 4 (GPX4) can protect cells from death triggered by diverse oxidative stress conditions, particularly those causing thiol deprivation[13–17], linking these early studies to the mechanism of ferroptosis.

Historically, cell death was considered to be passive and unregulated — until apoptosis was discovered in the 1970s as the first form of programmed cell death, as it is executed by a developmentally programmed pathway[18]. The broader category of regulated cell death refers to death programmes that are molecularly regulated, but not necessarily developmentally programmed[19]. We now know that ferroptosis meets this regulated cell death criterion: it is driven by lethal lipid peroxidation — a consequence of cellular metabolism and imbalanced redox

homeostasis — and can be suppressed by blocking lipid peroxidation directly or through depleting iron, via pharmacological or genetic means.

The field of ferroptosis is nonetheless nascent in many ways, having only coalesced from the adjacent fields of amino acid and lipid metabolism, iron homeostasis, redox and selenium biology, and cell death in the past few years. There has been an exponential growth in the number of published studies on ferroptosis, necessitating a thorough and critical look at recent advances to crystallize for diverse communities of researchers the key findings, questions and challenges surrounding this topic. Herein, we provide an in-depth analysis of the mechanisms and regulation of ferroptosis, its potential physiological functions and its role in disease and therapy. We discuss emerging questions and challenges that are conceptually important for the field. We also provide practical and experimental suggestions to guide ferroptosis research.

Mechanisms governing ferroptosis

Recent years have witnessed rapid progress in the mechanistic understanding of ferroptosis. Through the initial discovery of the role of the system x_c^-–GSH–GPX4 pathway in suppressing ferroptosis, the role of phospholipid hydroperoxides (PLOOHs) — a form of lipid-based reactive oxygen species (ROS) — as the executioners of ferroptosis is now established (FIG. 2). More recently, GPX4-independent ferroptosis surveillance pathways

have been identified. 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 have revealed an intimate relationship between ferroptosis and metabolic pathways.

The key role of GPX4

With the goal of discovering novel small-molecule anticancer therapies, the Stockwell group conducted a high-throughput screen beginning in 2001, leading to the publication, in 2003, of a series of compounds that were able to induce a unique form of non-apoptotic, non-necroptotic cell death[20]. Counter-screening revealed that multiple iron chelators and lipophilic radical-trapping antioxidants inhibited this type of cell death[21]. The requirement for iron in this cell death modality inspired coining the term 'ferroptosis'[1]. Subsequent mechanistic investigations identified two cellular components, system x_c^- and GPX4, inhibition of which by the compounds erastin and RSL3, respectively, induces ferroptotic death[1,22,23].

GPX4, a selenoprotein originally discovered by Ursini and colleagues through biochemical purification, is the major enzyme catalysing the reduction — and, thereby, detoxification — of PLOOHs in mammalian cells[24,25]. Reduction of phospholipid and cholesterol hydroperoxides to their corresponding alcohols by GPX4 requires the catalytic selenocysteine residue of GPX4 and two electrons provided most commonly by GSH, but also sometimes by other low-molecular thiols, or even protein thiols[26]. The thorough study of the first conditional *Gpx4* knockout mouse model by the Conrad group provided early evidence — before ferroptosis was recognized as a distinct cell death modality — that loss of *Gpx4* causes lipid peroxidation-dependent, non-apoptotic cell death in murine embryonic fibroblasts, and neurodegeneration in the hippocampus and cortical regions of the brain[17]. This and several other mouse models have helped to delineate a more detailed picture of the in vivo relevance of ferroptosis, as discussed further below.

As GPX4 is the major PLOOH-neutralizing enzyme, a general mechanism underlying erastin/RSL3-induced ferroptosis emerged: both compounds inactivate GPX4 — RSL3 does so directly, and erastin does so indirectly by inhibiting cystine import, thus depriving cells
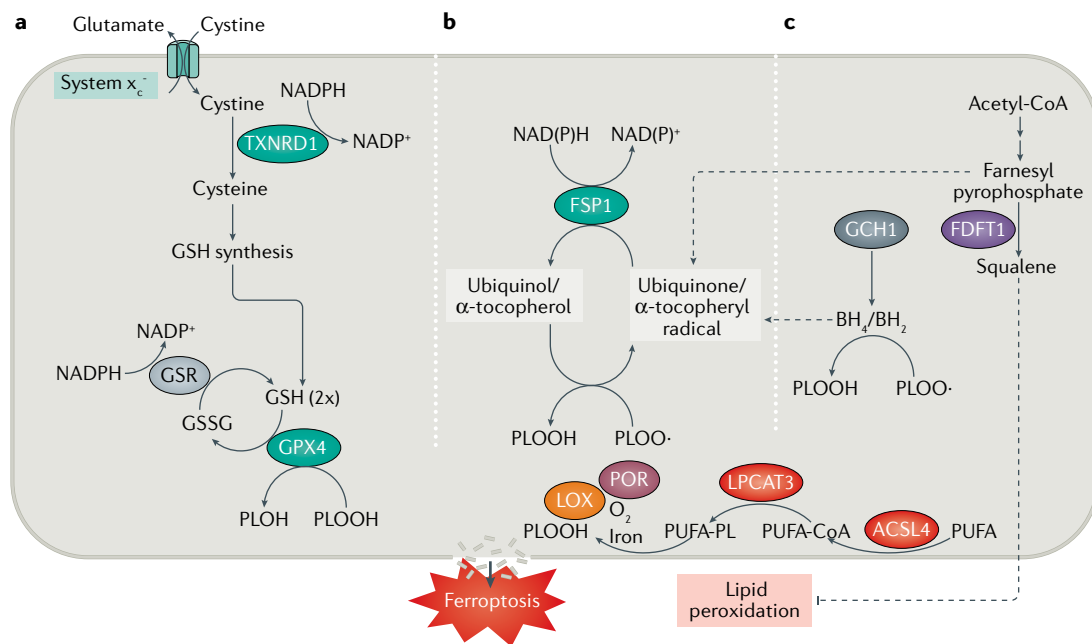

Fig. 2 | **Ferroptosis-suppressing pathways. a** | The canonical ferroptosis-controlling axis entails uptake of cystine via the system x_c^- cystine–glutamate antiporter, followed by glutathione (GSH) and/or thioredoxin reductase 1 (TXNRD1)-dependent reduction of cystine to cysteine and GSH biosynthesis. GSH is a potent reductant and a cofactor for glutathione peroxidase 4 (GPX4), thereby promoting GPX4-mediated reduction of any phospholipid hydroperoxides (PLOOHs) generated in the cell to yield the corresponding alcohols (PLOHs). Recycling of oxidized glutathione (GSSG) is achieved via glutathione–disulfide reductase (GSR) using electrons provided by NADPH/H⁺. **b** | Two independent genetic screens revealed that the ferroptosis suppressor protein 1 (FSP1)–ubiquinone system completely protects cells against ferroptosis induced by pharmacological inhibition or genetic deletion of GPX4. FSP1 prevents lipid peroxidation and associated ferroptosis via reduction of ubiquinone to ubiquinol (which, in turn, may directly reduce lipid radicals to terminate lipid autoxidation) and/or via regenerating the oxidized α-tocopheryl radical (vitamin E) to its non-radical form, which functions as the most powerful natural chain-breaking antioxidant in lipids. **c** | Alternate ferroptosis-suppressive mechanisms include squalene-mediated and di/tetrahydrobiopterin (BH₂/BH₄)-mediated inhibition of lipid peroxidation, likely by acting as endogenous radical-trapping antioxidants. ACSL4, acyl-CoA synthetase long-chain family member 4; FDFT1, farnesyl-diphosphate farnesyltransferase 1; GCH1, GTP cyclohydrolase 1; LOX, lipoxygenase; LPCAT3, lysophosphatidylcholine acyltransferase 3; PLOO•, peroxyl radical; POR, cytochrome P450 oxidoreductase; PUFA, polyunsaturated fatty acid; PUFA-PL, phospholipid containing polyunsaturated fatty acid chain.

of cysteine, an essential cellular building block of GSH. Consequently, PLOOHs accumulate, possibly causing rapid and unrepairable damage of membranes, leading to cell death (FIG. 2a). Conceptually, these findings established ferroptosis as a cell death modality with mechanisms distinct from other known death processes.

Drivers of phospholipid peroxidation

Unrestrained lipid peroxidation is the hallmark of ferroptosis. Early research in the 1950s pointed towards a nexus for the suppression of lipid peroxidation by the trace element selenium, as well as by vitamin E and cysteine[27,28]. Initiation of lipid peroxidation requires the removal of a bisallylic hydrogen atom (located between
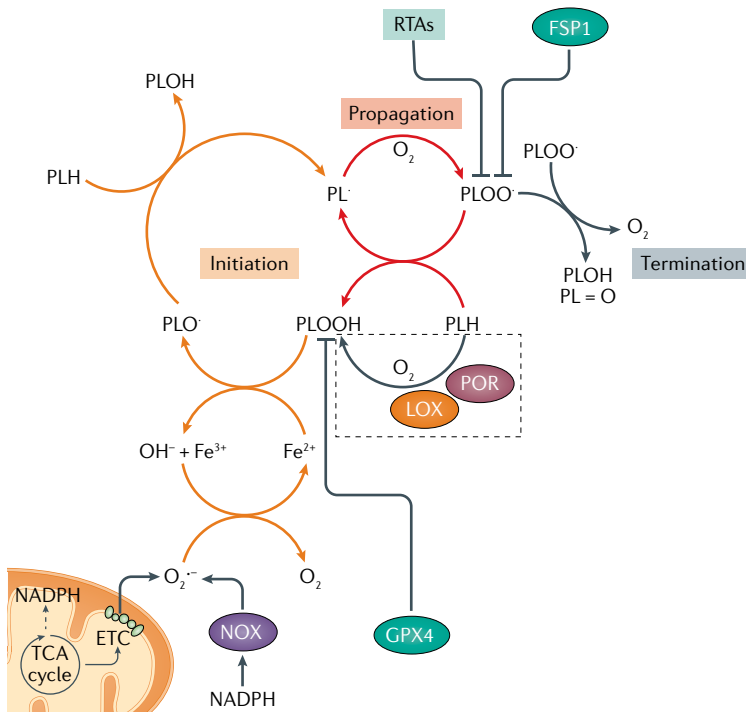

Fig. 3 | **Mechanisms of phospholipid peroxidation.** Lipid peroxidation, the hallmark of ferroptosis, can be divided into three phases: initiation, propagation and termination. The mechanisms initiating lipid peroxidation are not yet fully understood, but can potentially occur by both non-enzymatic and enzymatic processes (the latter marked by a dashed box). Non-enzymatic lipid peroxidation is, in theory, driven by the Fenton reaction, which utilizes iron and oxygen to catalyse a chain reaction, leading to the propagation of phospholipid peroxidation (formation of phospholipid hydroperoxides (PLOOHs)). Briefly, once the initial PLOOH is produced (through enzymatic processes or via free radicals generated in mitochondria and other cellular metabolic processes) and is not rapidly cleared by glutathione peroxidase 4 (GPX4), PLOOH can react with cellular labile iron to generate alkoxyl and peroxyl radicals, which lead to the propagation of PLOOH production, as exemplified in the figure. In the enzymatic mechanism of lipid peroxidation, lipoxygenases (LOXs) and/or cytochrome P450 oxidoreductase (POR) have been implicated to drive dioxygenation of lipids, although definitive genetic evidence for the involvement of lipoxygenases in the ferroptotic process is lacking. The lipid peroxidation-inhibiting systems (highlighted in green) — involving enzymes and small molecules — act on different levels of the lipid peroxidation cascade to prevent ferroptosis either by reducing phospholipid peroxides to corresponding phospholipid alcohols (GPX4) or by terminating radical-driven propagation (radical-trapping antioxidants (RTAs) and ferroptosis suppressor protein 1 (FSP1)). ETC, electron transport chain; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; $PL^{\bullet}$, phospholipid radical; PLH, phospholipid; $PLO^{\bullet}$, alkoxyl radical; $PLOO^{\bullet}$, peroxyl radical; PLOH, phospholipid alcohol; PL=O, phospholipid carbonyl; NOX, NADPH oxidase; OH^{-}, hydroxide ion; $O_2^{\bullet-}$, superoxide anion; TCA, tricarboxylic acid.

two carbon–carbon double bonds) from polyunsaturated fatty acyl moieties in phospholipids (PUFA-PLs) incorporated into lipid bilayers. This leads to the formation of a carbon-centred phospholipid radical ($PL^{\bullet}$), and subsequent reaction with molecular oxygen to yield a phospholipid peroxyl radical ($PLOO^{\bullet}$)[29,30], which removes hydrogen from another PUFA, forming PLOOH (FIG. 3). If not converted to the corresponding alcohol (PLOH) by GPX4, PLOOH and lipid free radicals — in particular, $PLOO^{\bullet}$ and alkoxyl phospholipid radicals ($PLO^{\bullet}$) — will react with PUFA-PLs to propagate PLOOH production by further hydrogen atom removal, reaction with molecular oxygen and formation of PLOOHs (FIG. 3). Eventually, this will lead to the formation of a myriad of secondary products, including breakdown products of lipid peroxides (such as 4-hydroxynonenal and malondialdeyde) and oxidized and modified proteins. This chain reaction may eventually lead to the breakdown of membrane integrity and, ultimately, to rupturing of organelle and/or cell membranes. Thus, membranes containing a high PUFA-PL content should be particularly vulnerable to peroxidation, which has been evidenced in neurons. The precise cellular membranes relevant to the lipid peroxidation that occurs during ferroptosis, such as membranes in mitochondria, endoplasmic reticulum, peroxisomes, lysosomes and the plasma membrane, are as yet unclear.

Genome-wide haploid and CRISPR–Cas9-based screens unveiled two membrane-remodelling enzymes, acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3)[31–33], as important drivers of ferroptosis. The role of ACSL4 in the ferroptotic process is based on its ability to ligate preferably long-chain PUFAs, including arachidonic acid (20:4) and adrenic acid (22:4), with coenzyme A. These products can then be re-esterified into phospholipids by various LPCAT enzymes, thereby increasing the cellular incorporation of long-chain PUFAs into lipids and membranes. Genetic loss of *ACSL4* or its pharmacological inhibition causes a dramatic shift from long-chain PUFA tails to short-chain and monounsaturated fatty acyl (MUFA) tails in phospholipids[31,34,35]. Such a dramatic change in the phospholipidome of ACSL4-deficient cells enables their proliferation upon *Gpx4* knockout for months, rescuing these cells from ferroptosis[31]. Along the same line, exogenous supplementation of MUFAs, stearoyl-CoA desaturase 1 (SCD1)-mediated cellular MUFA production and an ACSL3-dependent enrichment of membranes with MUFAs were reported to lower the cells' propensity to succumb to ferroptosis[36–38]. Notably, levels of expression of *ACSL4* in a subset of triple-negative breast cancer cell lines correlate with their sensitivity towards ferroptosis inducers[31], a correlation that seems to be shared with therapy-resistant, mesenchymal cancer cells[39] and clear cell renal carcinoma cells[33]. Suppression of *ACSL4* expression may thus be a principal mechanism in desensitizing cells to ferroptosis, which may be regulated by diverse signalling pathways, including signals from cell–cell and cell–extracellular matrix adhesions, which are both frequently affected in tumour development[40,41]. Conversely, increased expression

and/or activity of ACSL4 might promote ferroptosis under various pathophysiological contexts, such as ischaemia–reperfusion — a pathological tissue context that is strongly associated with ferroptosis induction — and response to radiation[42,43].

Although there is no doubt that the degree of unsaturation of lipid bilayers is key in determining the sensitivity of cells to ferroptosis, there are many uncertainties and debates on how lipid peroxidation is actually initiated. A bisallylic carbon separating a pair of dienes is one of the weakest C–H bonds known, and an increase in the number of these structures augments the rate of autoxidation of lipids, as is evident in the spoiling of PUFA-containing foods under ambient oxygen tension. Conceivably, non-enzymatic initiation of lipid peroxidation can be sparked by spontaneous generation of lipid free radicals or generation of the hydroxyl radical ($^{•}OH$) — one of the most reactive forms of ROS that attacks most of the organic molecules once produced locally (owing to its near diffusion-controlled rates) — likely driven by a Fenton reaction using iron as the catalyst[44] (FIG. 3) (detailed later).

Certain lipoxygenases (LOXs), which are non-haeme iron-dependent dioxygenases targeting PUFAs, can directly oxygenate PUFAs and PUFA-containing lipids in biological membranes[45], raising the prospect that LOXs may mediate ferroptosis induction. This possibility was supported by the observations that some pharmacological inhibitors of LOX can inhibit ferroptosis[17,46] and that *Alox15* knockout or application of the LOX inhibitor baicalein protected mice against ischaemic brain injury[47,48]. Nonetheless, genetic removal of *Alox15* on the *Gpx4* knockout background failed to prevent ferroptosis in mouse fibroblasts as well as to prevent acute ischaemic kidney injury and associated lethality in vivo[49], and neither did it restore the population of CD8+ T cells lost due to ferroptosis in T cell-specific *Gpx4*−/− mice[50]; of note, however, is that LOXs have been primarily implicated in ferroptosis induced by cysteine starvation, not ferroptosis induced by loss of GPX4, necessitating further dissection of the role of LOXs in ferroptosis using different models. These data suggest that alternative mechanisms may compensate for the loss of ALOX15 activity, that LOXs may only be involved in certain contexts of ferroptosis and/or that the frequently used 'LOX-specific' inhibitors exert non-specific activity as radical-trapping antioxidants and are able to prevent ferroptosis through this activity[51]. Indeed, a recent study confirmed that most frequently used LOX inhibitors harbour radical-trapping antioxidant activity[52], thus challenging a universal role of LOXs in ferroptosis. Further, combined downregulation of all human LOX isoenzymes failed to prevent RSL3-induced ferroptosis, although it provided substantial rescue against erastin-induced ferroptosis, likely because erastin treatment is associated with LOX enzyme activation[37]. Thus, LOXs may not be the key drivers of ferroptosis in most contexts but could contribute to the initiation and/or propagation of the damage in some contexts. In line with this, deletion of *Alox15* or *Alox12* showed protective roles in specific mouse models of neurodegeneration or cancer suppression[47,53], respectively. However, it should be stressed that these enzymes play important physiological roles in the immune system by directly modulating (neuro)inflammatory processes and the tumour microenvironment via generating pro-inflammatory and anti-inflammatory molecules[54,55]; thus, the beneficial effects of their loss may not necessarily be directly related to ferroptosis. Equally relevant to this notion are previous findings that GPX4 controls the activities of LOXs as well as activities of cyclooxygenases via the so-called cellular peroxide tone — that is, the sustained maintenance of low levels of peroxides in cells — as both of these enzymes require oxidation of their iron by lipid hydroperoxide to capture and incorporate molecular oxygen into PUFAs[54]. Notably, it has been reported that *ALOX12* is essential for p53-dependent ferroptosis triggered by peroxides, and this form of ferroptosis is rather unique as it appears to be independent of ACSL4 (REF.[53]); this implies that p53–ALOX12-driven ferroptosis may act through different lipids than ferroptosis driven by loss of GPX4 or cysteine starvation. In addition, phosphatidylethanolamine-binding protein 1 (PEBP1) has been reported to complex with certain LOXs, altering their substrate specificity towards PUFA-PLs[56].

Interestingly, despite the widespread lack of PUFAs in bacterial membranes, *Pseudomonas aeruginosa* expresses a secreted lipoxygenase (PA-LOX)[57]. This enzyme was shown to induce oxidation of membrane lipids of human red blood cells[58] and was able to induce ferroptosis in human bronchial epithelial cells[59]. Accordingly, patients with cystic fibrosis presented increased levels of oxidized arachidonic acid in phosphatidylethanolamine[59], which was reported to be one of the main phospholipid species detected in cells and tissues dying by ferroptosis[34]. Such a ferroptosis-inducing mechanism across organisms is intriguing and warrants further investigations to determine whether this mechanism is exploited by other lower organisms.

Besides the unclear role of LOXs in ferroptosis, recent findings suggest that the ubiquitously expressed cytochrome P450 oxidoreductase (POR) plays a role in initiating lipid peroxidation[60]. After accepting electrons from POR using NADPH as an electron donor, downstream electron acceptors, such as cytochrome P450 and CYB5A, are reduced, which could subsequently either directly or indirectly trigger lipid peroxidation by removing hydrogen from PUFAs or by reducing ferric iron (Fe^{3+}) to its ferrous form (Fe^{2+})[60,61] (FIG. 3); as described below, recycling between ferric and ferrous iron is crucial for the Fenton reaction and lipid peroxidation.

Iron in ferroptosis

As is evident from the name itself, ferroptotic cell death depends on iron. First, the non-enzymatic, iron-dependent Fenton chain reaction is likely essential for ferroptosis: when GPX4 is inhibited, PLOOHs can persist longer, initiating the Fenton reaction to rapidly amplify PLOOHs, the hallmark of ferroptosis[29] (FIG. 3). PLOOHs can react with both ferrous and ferric ions to generate the free radicals PLO$^{•}$ and PLOO$^{•}$, respectively, driving the damaging peroxidation chain reaction. It should be noted, however, that at least for the initiation phase of this iron-catalysed chain reaction, ferrous ions are likely to be more dominant, due

to the rather poor solubility and, thereby, bioavailability of ferric ions in cells. Furthermore, LOXs and POR require iron for catalysis. Iron is also essential for a plethora of redox-based metabolic processes that are involved in the generation of cellular ROS.

Given the central role of iron in cell viability and death, it is not a surprise that cellular iron homeostasis is under exquisite control, mainly through iron-regulatory proteins IRP1 and IRP2, which engage in post-transcriptional regulation of genes involved in intracellular iron storage/release and import/export[62,63]. Conceivably, many cellular processes alter the sensitivity of cells towards ferroptosis by changing cellular labile iron contents. For example, increasing cellular iron availability by autophagic degradation of the iron-storage protein ferritin promotes ferroptosis (see also next subsection)[64,65]. Similarly, transferrin and its receptor work together to promote ferroptosis by importing iron into the cell (see also next subsection)[66]. Conversely, mechanisms that enhance cellular iron export have been shown to render cells more resistant to ferroptosis[67,68]. Further, liberating iron via haeme-oxygenase 1 (HO-1)-mediated haeme degradation has also been implicated in ferroptosis; yet a series of conflicting data suggest HO-1 to either promote or suppress ferroptosis[69,70].

Recent in vivo mouse model studies further illustrated the role of iron regulation in ferroptosis. For example, genetic deletion of the ferritin heavy chain promotes cardiomyopathy, likely via enhancing ferroptosis[71]. Intriguingly, hepatocyte-specific knockout of the transferrin-encoding gene in mice led to a rather unexpected phenotype: feeding the knockout mice with an iron-enriched diet increased iron loading in hepatocytes — cells that normally synthesize transferrin in the body. This rendered the mice more susceptible to liver fibrosis, which could be ameliorated by lipophilic radical-trapping antioxidants[72], suggesting involvement of ferroptosis in this liver pathology. This study further showed that in the absence of transferrin expression, hepatocytes compensatorily upregulate the expression of a metal transporter SLC39A14, leading to excessive import of iron that then drives ferroptosis.

Metabolic inputs into ferroptosis
The role of iron, lipid species, ROS and cyst(e)ine in ferroptosis implies strong association between this cell death modality and cell metabolism. Studies by Jiang and colleagues seeking to determine how metabolism contributes to cell fate determination unravelled the intricate relationship of ferroptosis with metabolism[65,66,73]. The catabolic process of autophagy is a crucial survival mechanism in response to various stresses, but whether and how autophagy can also promote cell death (that is, 'autophagic cell death') has been debated for decades[74]. They found that upon amino acid starvation (a condition that triggers potent autophagy), autophagy promotes a rapid non-apoptotic, non-necroptotic form of cell death, but only if the medium was supplied with full serum. The iron-carrier transferrin and the amino acid glutamine in serum were found to be required for this form of cell death and the specific deprivation of cystine from the cell culture medium was sufficient to trigger

the death. Dependence on iron and the protective role of cystine pointed towards ferroptosis as a mechanism of cell death in these conditions[66]. The role of autophagy in cystine deprivation-induced ferroptosis is via autophagic degradation of ferritin (also known as ferritinophagy), which results in an increase of cellular labile iron content and thus sensitization to ferroptosis[64,65] (FIG. 4).

The requirement of glutamine metabolism, or glutaminolysis, for cystine deprivation-induced ferroptosis[66] links ferroptosis to oxidative metabolism. Glutamine is a key anaplerotic metabolite that fuels the mitochondrial tricarboxylic acid (TCA) cycle, thereby increasing the rates of mitochondrial respiration and augmenting the potential for the generation of ROS[66]. Hence, a normal metabolic function of mitochondria appears to be implicated in ferroptosis — a conclusion subsequently validated through various pharmacological, cellular and genetic analyses[73] (FIG. 4). Notably, mitochondria had been shown earlier to be active participants in oxytosis[6,75]. Besides mitochondria, plant cells possess another unique organelle that conducts a chain of oxidative–reductive anabolic reactions — the chloroplast. As ferroptotic cell death dependent on iron and ROS has been observed in plants[2,76–78], an intriguing speculation is that chloroplasts may also play an important role in the regulation of ferroptosis in plants.

Based on these findings, one can also raise a plethora of hypotheses and questions. For example, would glucose, a major fuel of the mitochondrial TCA cycle, regulate ferroptosis? Indeed, glucose starvation has been recently shown to suppress ferroptosis[79,80]. However, mechanistically this appears to be mainly due to signalling mediated by the key energy sensor AMP activated kinase (AMPK), instead of modulation of TCA and mitochondrial respiration (detailed later), suggesting multiple metabolic inputs into ferroptosis, which are currently elusive. On a related note, if the ferroptotic function of glutamine is solely through its role in supporting mitochondrial respiration, then how can glutamine be essential for cystine deprivation-induced ferroptosis even when cells are cultured in the presence of abundant glucose[66], which should alone support TCA activity and ensure high respiratory potential? Furthermore, and fundamental to ferroptosis, does mitochondrial metabolism promote ferroptosis by generating specific lipid precursors for PLOOH synthesis (intermediates of the TCA cycle can participate in lipogenesis) or via the generation of ROS (natural by-products of oxidative metabolic reactions)? The observation that both the mitochondrial activity and glutaminolysis are crucial for cysteine deprivation-induced ferroptosis, but are dispensable for that induced by GPX4 inhibition[73], lends more support to the latter possibility.

GPX4-independent surveillance pathways
Although the cyst(e)ine–GSH–GPX4 axis is considered the main system that opposes ferroptosis in mammals[22,49], genome-wide screens have recently uncovered GPX4-independent mechanisms of ferroptosis surveillance.

The first mechanism involves ferroptosis suppressor protein 1 (FSP1; also known as AIFM2)[81,82]. AIFM1 (REF.[83]), a homologue of FSP1 initially considered to be
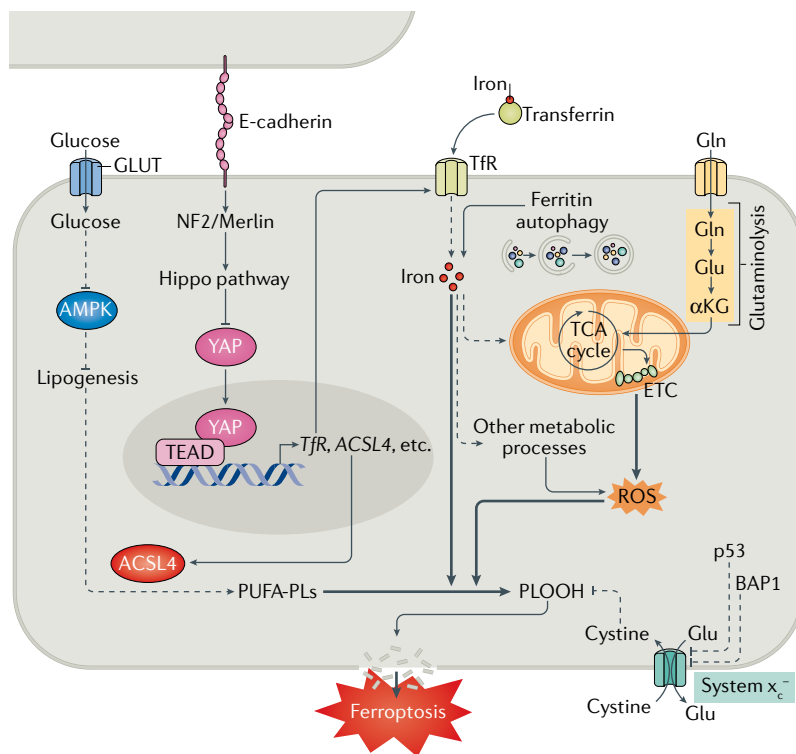
Fig. 4 | **Regulatory signalling in ferroptosis.** The regulation of ferroptosis by multiple metabolic events (such as lipogenesis, autophagy and the mitochondrial tricarboxylic acid (TCA) cycle) and signalling pathways (such as the E-cadherin–NF2–Hippo–YAP pathway, glucose-regulated AMP activated kinase (AMPK) signalling, and p53 and BAP1 tumour suppressor function). Lipogenesis involving production of phospholipids containing polyunsaturated fatty acid chains (PUFA-PLs) that is mediated by acyl-CoA synthetase long-chain family member 4 (ACSL4) and multiple other enzymes is required for phospholipid peroxidation and ferroptosis. Cellular iron is another essential factor of ferroptosis, thus processes such as transferrin receptor (TfR)-mediated iron import and autophagy-mediated ferritin degradation promote ferroptosis. Further, imbalanced cellular redox homeostasis contributes to ferroptosis. Therefore, when cells lack reducing agents such as cysteine, cellular metabolism — particularly oxidative metabolism in mitochondria — causes accumulation of reactive oxygen species (ROS) and promotes ferroptosis. The essential function of glutaminolysis in cysteine deprivation-induced ferroptosis can be partially explained by its anaplerotic role supporting the mitochondrial TCA cycle. Conceivably, multiple signal transduction pathways and transcription regulators alter ferroptosis sensitivity through modulating lipogenesis, iron homeostasis and cellular metabolism and redox homeostasis. For example, AMPK signalling prevents ferroptosis by mitigating lipogenesis, whereas glucose supports ferroptosis via antagonizing AMPK function; E-cadherin–NF2–Hippo signalling suppresses ferroptosis by attenuating YAP-mediated transcription of, among others, TfR and ACSL4; and tumour suppressors p53 and BAP1 sensitize cells to ferroptosis by suppressing the transcription of *SLC7A11* (a subunit of the system x_c^- cystine/glutamate antiporter) and thus reducing the import of reducing agent cystine. ETC, electron transport chain; Gln, glutamine; Glu, glutamate; αKG, α-ketoglutarate; PLOOH, phospholipid hydroperoxide.

FSP1 suppresses lipid peroxidation and ferroptosis by either reducing ubiquinone (or its partially oxidized product semihydroquinone) to yield ubiquinol, which in turn may directly reduce lipid radicals to terminate lipid autoxidation, or indirectly via regenerating oxidized α-tocopheryl radical (vitamin E)[81,82] — a powerful natural antioxidant (FIG. 2b). This protective role of ubiquinone sheds light on a long-standing mystery of why some cells and tissues, such as highly metabolically active hepatocytes[88], contain a large pool of extramitochondrial ubiquinone, which is inconsistent with its canonical role in the mitochondrial electron transport chain.

In another study, GTP cyclohydrolase 1 (GCH1) was reported to protect against ferroptosis via its metabolic products tetrahydrobiopterin (BH4) and dihydrobiopterin (BH2)[89]. BH4 was shown to confer protection of phospholipids containing two PUFA tails against oxidative degradation, likely involving a dual mechanism: by acting as a direct radical-trapping antioxidant and being involved in ubiquinone synthesis[89,90] (FIG. 2c). Although the role of GCH1 in protecting tissues and organs from ferroptosis remains to be elucidated, knockout studies showed that loss of *Gch1* in mice causes bradycardia and embryonic death during mid-gestation[91].

Besides these systems that either directly act on peroxides in lipid bilayers or on phospholipid radicals via naturally occurring radical-trapping antioxidants, other cell-intrinsic mechanisms may exist that protect against deleterious lipid peroxidation. In this context, accumulation of squalene, a metabolite of the cholesterol pathway, was reported to confer anti-ferroptotic activity in cholesterol-auxotrophic lymphoma cell lines and in primary tumours, although it remains to be shown whether this is a cancer subtype-specific effect or a general protective mechanism[92] (FIG. 2c).

Regulation of ferroptosis

Conceivably, biological processes that modulate ferroptosis-promoting or surveillance molecules, redox and iron homeostasis, and cell metabolism could impact ferroptosis. As expected, the oxidative-stress-responsive transcription factor NRF2 can mitigate ferroptosis by stimulating the expression of its multiple canonical target genes (see REF.[93] for a comprehensive review). Additionally, mounting evidence has demonstrated that, under specific biological contexts, multiple signalling pathways can dictate the susceptibility of cells to ferroptosis.

Regulation of ferroptosis suppressors

Despite the prevailing importance of GPX4 and FSP1 for limiting ferroptosis, little is known with regard to how cells regulate their anti-ferroptotic potential under both physiological and pathological conditions. Nevertheless, it seems that both GPX4 and FSP1, at least to some extent, intersect with the mevalonate pathway: one of the outputs of this pathway, isopentenylation, stabilizes the selenocysteine-specific tRNA (*Trsp*), which is required for the synthesis of selenoenzymes including GPX4, and as one of the final metabolites of the mevalonate pathway, ubiquinone, is a major substrate of FSP1, as discussed above[94].

Ubiquinone
A lipophilic metabolite (also known as coenzyme Q10) generated by the mevalonate pathway that widely acts by shuttling electrons in the mitochondrial respiratory chain.

Mevalonate pathway
A lipid biosynthesis pathway that produces terpene-derived compounds such as cholesterol and ubiquinone.

pro-apoptotic (like FSP1/AIFM2 (REFS[84,85])), is nowadays associated with transport and proper folding of mitochondrial intermembrane proteins[86]. Similarly, FSP1 lacks substantial pro-apoptotic function, but in fact protects cells from ferroptosis induced by inhibition or genetic deletion of *GPX4* (REFS[81,82]). FSP1 is myristoylated and associates with several cell membrane structures, including the plasma membrane, Golgi apparatus and perinuclear structures. Mutation of the myristoylation site abrogates its anti-ferroptotic function. Mechanistically, due to its NADH:ubiquinone oxidoreductase activity[87],

GPX4 is one of the 25 selenoproteins in humans, and hence its expression is regulated by cellular selenium availability[94,95]. For instance, selenium supplementation was shown to boost GPX4 expression in neurons via coordinated activation of the transcription factors TFAP2c and Sp1, which protected against tissue damage in a haemorrhagic stroke mouse model[96]. However, it should be noted that GPX4 can be regarded as a housekeeping protein, being constitutively expressed in most tissues and organs, unlike true selenium-responsive proteins, such as selenoprotein P (SELENOP), GPX1 and GPX3. Other transcription factors that have been reported to regulate GPX4 expression include C/EBPα in enterocytes[97] and NF-Y in some cancer cells[98]. Post-transcriptionally, guanine-rich sequence-binding factor 1 (GRSF1) was reported to bind to the 5′ untranslated region of the mitochondrial form of the *Gpx4* mRNA, causing increased translation of mitochondrial GPX4 that plays an essential role during sperm development[99,100]. The relevance of these regulatory events to ferroptosis, however, remains undefined.

Accumulating evidence suggests that GPX4 is regulated on the activity and stability level as well. For instance, impaired GSH-dependent reduction of the active site selenocysteine — due to sustained oxidative stress and concomitant lack of GSH — may cause irreversible inactivation of GPX4 by formation of a redox-dead dehydroalanine in a process known as β-cleavage[101]. Low levels of GSH may also trigger the formation of a selenylamide between the selenenic acid and a neighbouring amino acid, thereby protecting the enzyme from irreversible inactivation under acute oxidative stress conditions. However, it needs to be further explored whether any of these mechanisms are at play during pathological conditions, such as ischaemia–reperfusion injuries; it has been reported that intestinal ischaemia is associated with much lower GPX4 activity and levels[42]. In addition, several well-established ferroptosis inducers, including RSL3, ultimately cause impaired activity, synthesis and stability of GPX4 through mechanisms that include covalent modification of the active site selenocysteine, disruption of the mevalonate metabolism and general iron-dependent oxidative stress[102–104].

As a prominent ferroptosis suppressor, FSP1 was originally described as a p53-responsive gene and therefore initially called p53-responsive gene 3 (PRG3)[105]. FSP1 is a target of transcription factors NRF2 (REF.[106]), CRBP[107] and PPARα[108]. Interestingly, in T cell lymphoblastic lymphoma cells, FSP1 expression was reported to be upregulated by the long non-coding RNA maternally expressed 3 (MEG3) and suppressed by miR-214 (REF.[109]), two regulatory RNAs involved in tumour development. Beyond transcriptional regulation, almost nothing is known about how the oxidoreductase activity of FSP1 is regulated and how its subcellular localization impacts on its role in different physiological and pathophysiological processes[81,82,84,107]. But the promiscuity of FSP1 towards both the reducing and oxidizing substrates (including NADH, NADPH, ubiquinone and α-tocopherol) suggests complex regulation of its activity.

Hippo–YAP signalling in ferroptosis

The Hippo–YAP pathway is involved in a myriad of biological functions, including cell proliferation and organ size control[110,111]. The investigation of the role of this pathway in ferroptosis was initiated from an observation that cells grown at high density are often more resistant to ferroptosis induced by both cysteine deprivation and GPX4 inhibition[41]. This observation is reminiscent of earlier reports, importantly including demonstration that culturing at extremely high density or as spheres (where cell–cell contacts are favoured) promotes survival of *Gpx4* knockout cells[17,112]. Mechanistically, the cell density effect on ferroptosis in epithelial cells is mediated by E-cadherin-mediated cell–cell contacts, which activate Hippo signalling — through NF2 (also known as Merlin) tumour suppressor protein — and thus inhibit nuclear translocation and the activity of transcriptional co-regulator YAP. Because YAP targets several regulators of ferroptosis, including ACSL4, transferrin receptor TfR1 and possibly others, ferroptosis susceptibility will inevitably depend on the activity of the Hippo pathway, with increased susceptibility upon Hippo suppression and YAP activation[41] (FIG. 4). Consistent with this finding, TAZ, the close homologue of YAP, has also been shown to enhance ferroptosis in a cell density-regulated manner in kidney cancer cells that predominantly express TAZ instead of YAP[113].

The role of the E-cadherin–NF2–Hippo–YAP/TAZ pathway in dictating ferroptosis sensitivity has important implications. First, as multiple components of this pathway are frequently mutated in cancer, mostly leading to increased YAP/TAZ expression and/or activity, ferroptosis induction might be exploited as a potential therapeutic approach for the treatment of these specific cancers, a topic further discussed below. Second, as such cell density-dependent ferroptosis was also observed in non-epithelial cells that do not express E-cadherin[41], it should be considered that other cadherins or cell adhesion molecules may also mediate similar mechanisms for ferroptosis suppression. Thirdly, the Hippo–YAP pathway is important in development and interacts with various other signalling pathways, thereby providing a plethora of potential links between ferroptosis and normal cell biology. Finally, it is tempting to speculate that the ancestral function of cadherins — expression of which can be traced all the way back to some primitive metazoan species[114] — could be to protect cells from oxidative stress challenges and its most devastating consequence, ferroptosis.

AMPK signalling and ferroptosis

Intuitively, energy and metabolic stress should result in the loss of energy and, thus, a cascading failure of systems needed to maintain homeostasis, such as energy-dependent ion gradients across cell membranes[115], ultimately resulting in cell death. Moreover, metabolically stressing cells with glucose starvation increases ROS production[116], suggesting that glucose starvation promotes ferroptosis. Surprisingly, glucose starvation blocks ferroptosis[79,80]. This protective effect was found to depend on the activity of energy-sensing kinase, AMPK. Hence, when glucose is absent, AMPK is

activated, turning on an energy stress-protective pro-gramme against ferroptosis that involves impaired biosynthesis of PUFAs, which are essential for lipid peroxidation-driven ferroptosis[34,37] (FIG. 4). This find-ing has practical applications, as activating this energy stress programme was found to protect against renal ischaemia–reperfusion injury[79]. More generally, this protective mechanism may exist as a first line of defence against organ injury, which is presumably associated with energy failure.

Hypoxia signalling and ferroptosis

Given that ferroptotic cell death is driven by phospho-lipid peroxidation, a long-standing question has been whether it is dependent on oxygen concentrations, as molecular oxygen can be metabolized by various enzymes first to the superoxide anion (O^-), which then undergoes dismutation forming hydrogen peroxide (H_2O_2). However, one of the early studies of ferropto-sis indicated little loss of sensitivity to erastin-induced ferroptosis in 1% oxygen, suggesting that hypoxia does not suppress ferroptosis[23]. In fact, there is evidence that hypoxia promotes ferroptosis. First, it should be considered that hypoxia is associated with increased production of ROS by mitochondrial complex III, which could directly contribute to lipid peroxidation by damaging mitochondrial membranes, lead to ele-vated cellular levels of H_2O_2 to support Fenton reac-tions and/or act by overwhelming the antioxidant capacity of the cell. Further, hypoxia drives the activa-tion of hypoxia-inducible factors (HIFs), and a recent study of clear cell carcinoma found that the high sen-sitivity of these cells to ferroptosis induced by GPX4 inhibition is driven by the HIF2α isoform. In this case, HIF2α was shown to be responsible for the expression of hypoxia-inducible, lipid droplet-associated protein (HILPDA), which then drove the enrichment of poly-unsaturated lipids[33]. Clear cell carcinomas have a char-acteristic clear cytoplasmic staining upon histology staining and are difficult to treat[117], indicating a clin-ical implication of this finding. Also, it is tempting to speculate that the HIF2α–HILPDA-driven sensitization to ferroptosis may be an ancient means to eliminate nascent hypoxic tumours (see also next section).

Biological functions of ferroptosis

The mechanisms of ferroptosis suggest its potential evolutionary origin: ever since living organisms on Earth began to use oxygen to drive metabolism, essen-tially through a series of chemical redox reactions, the transition metal iron has become the prime factor to catalyse these reactions. An inevitable side product of such iron-dependent, redox-based metabolism is ROS, including PLOOHs. When the cellular level of PLOOHs exceeds a certain threshold, cells succumb to ferropto-sis. Conceivably, multiple surveillance mechanisms such as GPX4 and FSP1 have evolved to protect cells from ferroptosis. Thus, speculatively, ferroptosis might be a most ancient form of regulated cell death emerging in the environment with abundant iron and oxygen.

But is there any beneficial, physiologically relevant role for ferroptosis? In theory, this is possible. A related example might be ROS and lipid hydroperoxides. These species were first considered as mere toxic 'by-products' of metabolism, but we now know that they confer essen-tial physiological functions, for instance in cell signalling and immunity, and that they are deliberately generated at several subcellular sites to regulate cellular physio-logical events[118–120]. Similarly, ferroptosis might also be adapted to accomplish roles beneficial for life. Mounting evidence, although indirect, suggests the physiologi-cal function of ferroptosis in tumour suppression and immune surveillance.

Ferroptosis in tumour suppression

Multiple tumour suppressors have been shown to sensi-tize cells to ferroptosis. Therefore, a reasonable hypoth-esis is that ferroptosis contributes to the antitumour activity of these tumour suppressors; that is, tumour suppression might be an innate physiological function of ferroptosis.

Among such tumour suppressors, the involvement of p53 in ferroptosis has been thoroughly investigated. Through a detailed analysis of specific lysine acetyl-ation sites of p53, it was found that p53 can poten-tiate ferroptosis via suppressing the transcription of system x_c^- subunit *SLC7A11*, and this function may contribute to the tumour-suppressive function of p53 in vitro and in vivo[121,122]. Furthermore, a cancer-prone single-nucleotide polymorphism of p53, leading to P47S amino acid substitution, was found to confer resistance to ferroptosis in cancer cells[123]. However, it is not clear whether the loss of ferroptosis-promoting activity of p53 is the only functional consequence caused by these spe-cific mutations. Notably, in contrast to these studies, p53 has also been reported to prevent ferroptosis via mod-ulating its other transcriptional targets[124,125]. Given that p53 can regulate a large cohort of target genes that are involved in various biological processes, its precise role in ferroptosis could well be context-dependent.

Similar to p53, the tumour suppressor and epigenetic regulator BAP1 can also promote ferroptosis by down-regulating *SLC7A11* expression[126]. But unlike p53, whose ferroptosis-promoting activity alone has been suggested to be sufficient to suppress tumorigenesis in vivo[122], it is not clear how significantly the ferroptosis-promoting activity of BAP1 contributes to its tumour-suppressive function.

Fumarase, an enzyme catalysing the conversion of fumarate to malate in the TCA cycle, is a bona fide tumour suppressor in leiomyoma and papillary renal cell carcinoma[127]. It has been elusive how this enzyme, essen-tial for TCA cycle activity and, hence, cellular energy and metabolite generation, can counter-intuitively suppress tumorigenic growth, and how it does so only in a few specific cancer types. The involvement of fumarase in the ferroptosis-promoting function of the mitochondrial TCA cycle[73] sheds light on these questions. Development of cancer is frequently associated with increased mito-chondrial ROS production, which primes these cells for ferroptosis. Loss of fumarase function will impinge on oxidative function of mitochondria, which on the one hand will impair the growth capability but, on the other, will render cells more resistant to ferroptosis, thus

enhancing their survival and potential for cancerous transformation.

Ferroptosis in immune surveillance

Unlike other forms of regulated cell death such as apoptosis, pyroptosis and necroptosis, which are clearly engaged by the immune system to regulate immune responses[128], it remains largely obscure whether sensitizing or triggering ferroptosis by extrinsic or intrinsic mechanisms may fulfil a similar 'physiological role'. There is some preliminary evidence showing that ferroptosis could be important in inducing cell death by immune cells. One report suggested that the expression of system x_c^- is suppressed by interferon-γ (IFNγ)[129], and the production of this cytokine by CD8+ T cells was recently shown to be involved in sensitizing tumour cells towards ferroptosis[130]. Whether such a direct mechanism of immune cells on ferroptosis induction might be of physiological relevance remains elusive. Another report indicated that IL-4 and IL-13 suppress GPX4 expression in certain cells (including the kidney, lung, spleen and heart) coinciding with increased expression of ALOX15, thus permitting a robust production of arachidonic acid metabolites — key inflammatory intermediates[131]. As GPX4 is known to suppress the activities of LOXs and cyclooxygenases by lowering the lipid peroxide tone[54], it is possible that impaired activity of GPX4 may have a profound effect on the secretion of immunomodulatory lipid mediators, which in turn may inform the immune system about the presence of cells in a ferroptosis-sensitive state, thereby contributing to immune surveillance (detection of damage or malignancy).

The potential function of ferroptosis in immune responses is not limited to the mammalian system and could be relevant to plants as well. A recent report suggests that in rice, induction of ferroptosis-like cell death — associated with lipid peroxidation and subject to inhibition by iron chelators — prevents infection by fungus *Magnaporthe oryzae* by removing infected cells and preventing the pathogen from spreading[4]. Intriguingly, ferroptosis-like death of specific cells of *M. oryzae* is

conversely required for its development in the host. As such, this finding also implicates, for the first time, a potential developmental role of ferroptosis.

Implications of ferroptosis in disease

Although the contribution of ferroptosis to physiology remains obscure, its role in a plethora of human pathological states has been extensively documented. In this section, we will focus on the roles of ferroptosis in cancer and ischaemia–reperfusion injuries, which have been by far the best studied. Importantly, pharmacological modulation of ferroptosis has been demonstrated to be a promising therapeutic venue for the treatment of these conditions in diverse preclinical animal models. Nevertheless, the links between ferroptosis and other diseases are also emerging as discussed in BOX 1, suggesting that ferroptotic cell death has broad implications for health.

Ferroptosis in cancer

Ferroptosis has been linked to cancer since the very beginning of the field: the initial discovery of chemical inducers of ferroptosis is the result of hunting for novel cancer therapeutic compounds[20,21]. Subsequent mechanistic studies have revealed that numerous cancer-relevant genes and signalling pathways regulate ferroptosis, as discussed in previous sections. On the one hand, it has been observed that mesenchymal and dedifferentiated cancer cells, which are often resistant to apoptosis and common therapeutics, as well as so-called 'therapy-persister' cancer cells are highly susceptible to ferroptosis inducers[39,132,133]. Conceptually, as ferroptosis is an oxidative-stress-induced form of cell death that is tightly interwoven with cell metabolism, it seems logical to propose that cancer cells may have higher tendency to undergo ferroptosis, due to overall more active metabolism and higher ROS load. Adding to this notion, it has been shown that cancer cells often demand high iron supply[134,135], which may further sensitize them to ferroptosis. However, cancer cells may also harness additional genetic or epigenetic mechanisms to counter these metabolic and oxidative burdens, such as increased expression of SLC7A11 or upregulation of the antioxidative transcription factor NRF2 (REF.[136]), reducing their susceptibility to ferroptosis. Therefore, whether a given cancer is more sensitive or resistant to ferroptosis induction is dictated by its specific genetic background. The genomics of cancer, as well as various other parameters as discussed below, should be considered for the development of ferroptosis induction-based cancer therapy.

Ferroptosis sensitivity of cancer cells. Multiple oncoproteins, tumour suppressors and oncogenic signal transduction pathways can regulate ferroptosis. Therefore, their alterations in cancer can be used as biomarkers to predict the responsiveness of cancer cells to ferroptosis-inducing therapies.

Taking the E-cadherin–NF2–Hippo–YAP pathway as an example and data from The Cancer Genome Atlas (TCGA): loss of function mutation of tumour suppressor E-cadherin is a frequent event in breast lobular invasive carcinoma (~65%) and diffusive gastric adenocarcinoma

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Box 1 | Potential association of ferroptosis with pathology

In addition to cancer and ischaemic organ injuries, ferroptosis has been implicated in the pathogenesis of a growing list of other diseases, such as neurodegeneration[17,164–166,181], liver and lung fibrosis[72,182], autoimmune diseases[183,184], *Mycobacterium tuberculosis*-induced tissue necrosis[185], cigarette smoking-associated chronic obstructive pulmonary disease[186,187] and the rare genetic neurological disorder Pelizaeus–Merzbacher disease[188]. Although this long list speaks to the clinical relevance and therapeutic potential of ferroptosis-modulating approaches, further investigation is required to determine whether there is indeed a causative role of ferroptosis in these diseases. For example, in most cases, the general observations were that non-apoptotic cell death was induced in the disease tissue and that a ferroptosis inhibitor, often a lipophilic radical-trapping antioxidant, could mitigate the observed cell death and, in some cases, alter the severity of symptoms. However, lipid peroxides can regulate immunity and inflammation, processes that play important roles in all of the listed diseases. Therefore, caution is needed to distinguish whether the observed effect of lipophilic radical-trapping antioxidants occurs via modulation of inflammation or ferroptosis, or both. A detailed interrogation of mechanisms of cell death involved, including examining specific in vivo ferroptosis biomarkers (which the field sorely miss), will be crucial to clarify the contribution of ferroptosis to these pathologies.

(~25%), among other cancers; loss of function mutation of NF2 occurs in >30% of mesothelioma and in all of a group of benign diseases known as NF2 diseases; and, similarly, mutation of Hippo component LATS1/2 tumour suppressors also occurs in various cancers. Although genetic mutation of YAP is rare, its overexpression and post-translational activation are frequently observed in cancer. Importantly, malignant mutations of these genes usually drive metastasis, protect cancer cells from apoptosis and make them more resistant to common cancer therapies[137,138]. Therefore, the finding that these same mutations sensitize cancer cells to ferroptosis[41] unveils an unusual 'Achille's heel' of these malignant cells and suggests a unique therapeutic opportunity of ferroptosis induction. This opportunity is particularly attractive for gastric cancer and mesothelioma, both of which currently lack effective treatment[139,140]. In addition to mutations in the E-cadherin–NF2–Hippo–YAP pathway, we expect that more biomarkers guiding ferroptosis-inducing cancer therapy will be identified in the near future.

Potential ferroptosis-inducing anticancer therapies.
The development of ferroptosis induction-based cancer therapies is being actively pursued. In this space, several untargeted nanoparticle-based strategies to deliver iron, peroxides and other toxic cargoes to kill tumour cells have been tested. Further, the presence of multiple enzymes that control ferroptosis enables the development of targeted approaches[141,142]. Perhaps the most obvious target is GPX4, as it is expressed in most cancer cell lines and is important for their survival[22]. Yet GPX4 lacks a classical small-molecule binding pocket, and the available GPX4 inhibitors covalently modify the selenocysteine residue of GPX4 as well as other selenoproteins[143], raising the issue of specificity and potential toxicity. These inhibitors are also highly reactive and thus unstable, but this may be overcome by developing masked prodrugs that can be metabolically converted into their active forms intracellularly[144,145]. Nonetheless, the main caveat remains that GPX4 is essential for various peripheral tissues, such as kidney tubular cells and certain neuronal subpopulations in mice[29], so targeting GPX4 will probably cause substantial side effects unless the therapeutics could be delivered specifically to the tumour cells.

Unlike targeting GPX4, approaches to limit cellular cyst(e)ine availability by inhibiting system x_c^- are highly promising given that *Slc7a11* knockout in mice does not cause major pathologies[146] and that the expression of SLC3A2 and/or SLC7A11 negatively correlates with clinical outcome of patients with melanoma and glioma[130,147]. Indeed, studies to restrain tumour growth and tumour metastasis in various types of cancer by inhibiting system x_c^- either pharmacologically[148,149] or genetically[150–156] have provided highly promising results in mouse models, showing both efficacy and low toxicity. The higher vulnerability of various tumours to system x_c^- inhibition than that of normal tissues is likely due to the more active metabolism and other alterations in tumour cells, making them subjective to sustained oxidative stress and, thus, more dependent on system x_c^- function

for detoxification of ROS. Obviously, in order to apply system x_c^--inhibition-based therapies, a careful stratification of patient tumour tissues is required to examine system x_c^- expression (for example, SLC7A11 overexpression may indicate cancer cell addiction to cystine for ROS scavenging)[82] and other biomarkers as discussed in the previous subsection that determine the sensitivity of tumours to system x_c^- inhibition.

Similar to the loss of *Slc7a11*, knockout of *Fsp1* does not cause embryonic lethality or apparent pathologies[157], suggesting a broad therapeutic window for targeting FSP1. Moreover, FSP1 is abundantly expressed in a large number of cancer cell lines and is the highest ranked gene correlating with the resistance to GPX4 inhibitors in a panel of 860 cancer cell lines[82]. Cancer cells deprived of GPX4 can be efficiently killed by FSP-specific inhibitor iFSP1, whereas in GPX4-proficient cancer cells iFSP1 synergizes with RSL3 to induce ferroptosis[82]. Therefore, FSP1 inhibitors may find their way into the clinics, especially for therapy-resistant tumours or tumours showing characteristics of dedifferentiation.

Ferroptosis induction-based treatment might also be combined with other therapeutic approaches, with immune checkpoint blockade and radiotherapy as potential options. Combining immune checkpoint blockade with other therapies has been actively pursued. One rationale is that these other therapies may induce immunogenic cell death in tumour tissues to enhance the potency of immune checkpoint blockade. Intriguingly, there is recent evidence showing that immune checkpoint blocking therapy with anti-PDL1 antibodies can potentiate ferroptosis-inducing therapy[130]. Specifically, anti-PDL1 antibodies stimulated CD8+ T cells to secrete IFNγ, which, as mentioned above, suppresses system x_c^- activity in target cancer cells, thus sensitizing them to ferroptosis. Therefore, immunotherapy in combination with ferroptosis induction represents a promising treatment in that the two therapeutic modalities mutually potentiate each other, leading to a synergistic anticancer effect. As regards radiation, recent evidence indicates that it can induce ferroptosis on its own, and can also synergize with ferroptosis inducers and immunotherapy, providing potentially effective therapeutic combination regimens[43,158,159]. This sensitizing effect was observed at the level of cell death, lipid peroxidation, ferroptosis-linked gene expression changes and lipidomic changes, as well as in cell-line and patient-derived xenograft cancer models and freshly isolated patient glioma slice cultures. Moreover, cytoplasmic but not nuclear radiation was found to synergize with ferroptosis inducers, suggesting that in these therapeutic regimens radiation promotes cell death via its role in depleting GSH and induction of lipid peroxidation, rather than its canonical DNA-damaging effects[158]. Increased sensitivity to ferroptosis has also been achieved by the combination of radiation and immunotherapy. In this case, irradiation synergized with immunotherapy in downregulating *SLC7A11*, mediated by DNA damage-activated kinase ATM and IFNγ[159]. Finally, radiation has been shown to upregulate ACSL4 in cancer, resulting in elevated lipid peroxidation and ferroptosis[43]. The mechanisms involved here are not clear but could involve activation of transcription

Immune checkpoint blockade
The process of activating immune cells by inhibiting a signalling pathway that normally suppresses their activity; therapeutically, inhibiting cancer cell immune checkpoint enables the human immune system to eliminate cancer cells.

regulators that mediate both radiation response and ferroptosis, such as p53 and BAP1. Therefore, the ferroptosis effect of radiation is likely mediated by multiple mechanisms and may be context-dependent. Notably, ferroptosis is implicated as a contributor to some of the adverse events of radiation, such as lung fibrosis and killing of granulocyte–macrophage haematopoietic progenitor cells[160,161].

Ferroptosis in ischaemic damage

Ischaemia followed by reperfusion can induce massive cell death and inflammatory response in the affected organs, resulting in devastating diseases including brain stroke, ischaemic heart disease and injuries to the liver and kidney. Remarkably, ischaemic heart disease remains the disease that causes highest mortality worldwide[162]. Strong evidence, as detailed below, indicates that ferroptosis is a major contributor to cell death associated with ischaemia–reperfusion injuries, due to, at least partially, oxidative stress induced by ischaemia. These findings suggest that ferroptosis inhibition is a potential therapeutic approach for the treatment of conditions associated with ischaemic damage.

Contribution to ischaemic cell death in the brain and the heart. Multiple studies have established a role of ferroptosis in neuronal demise, including stroke and other brain injuries. An ex vivo experiment using rat hippocampal slice culture showed that glutamate-induced neuronal excitotoxic cell death can be blocked by radical-trapping antioxidant ferrostatin 1 (REF.[1]). As glutamate-induced neurotoxicity is involved in stroke and various neurodegenerative diseases[163], and a high concentration of extracellular glutamate can induce ferroptosis through inhibiting system x_c^- function[1], ferroptosis probably contributes to the pathogenesis of these brain diseases. Consistently, genetic studies in mice confirmed that conditional *Gpx4* deletion can cause symptoms mimicking neurodegeneration[17,164–166]. Further, iron chelators and lipophilic radical-trapping antioxidants have been tested for mitigating stroke and neurodegeneration in diverse experimental systems[67,165,167,168].

The role of ferroptosis in ischaemic heart disease has also been extensively investigated. In an ex vivo system mimicking ischaemia–reperfusion injury of the mouse heart, it has been shown that iron chelators and glutaminolysis inhibitors significantly mitigated cardiomyocyte cell death, reduced damage of heart tissue and improved its function, suggesting the potential therapeutic value of ferroptosis-targeting for the treatment of ischaemic heart disease[66]. More recently, an in vivo mouse model study further confirmed this notion[169].

To design feasible ferroptosis-targeted therapies to treat the fatal diseases of stroke and ischaemic heart injury, many important factors need to be considered. For example, both diseases can be extremely acute and only allow a short time window for intervention; therefore, can ferroptosis-targeted therapies be applied timely, thereby exerting their effects rapidly enough? Moreover, as there are multiple ferroptosis surveillance pathways, and ischaemia–reperfusion may induce ferroptosis in different organs via selectively disrupting one of these

surveillance pathways, could we develop specific ferroptosis inhibitors that can effectively treat a certain disease while causing minimal side effects to other organs?

Roles in ischaemia–reperfusion injury associated with organ transplantation. Besides the brain, perhaps the kidney is the most ferroptosis-sensitive organ identified in adult mammals; survival of the proximal kidney tubular cells depends on functional GPX4 (REF.[49]), and these cells are susceptible to cell death in response to an ischaemia–reperfusion scenario, which is a common complication of kidney transplantation. Consistently, ferroptosis inhibitors have been shown to mitigate kidney tubular cell death and acute renal failure in mouse models of ischaemia–reperfusion injury, in the genetic model of inducible whole-body *Gpx4* deletion and in folic acid-induced acute kidney injury[49,170,171]. These studies also suggest that ferroptosis causes activation of the innate immune system owing to a pro-inflammatory nature of this cell death, which could further compromise the success of transplantation by mediating transplant rejection[171–173]. Another frequently transplanted organ is the liver. Whereas mice with hepatocyte-specific ablation of *Gpx4* die neonatally, high dietary vitamin E can compensate for the lack of GPX4 in the liver[174]. Moreover, the use of radical-trapping antioxidants protects the liver parenchyma from ischaemia–reperfusion injury[49]. Lastly, ferroptosis has also been implicated in heart transplantation[175]. Collectively, ferroptosis inhibitors might be effective drugs to support the success of transplantation of various organs.

Potential targets for blocking ferroptosis in ischaemia–reperfusion injury. There are several potential points of therapeutic intervention in the ferroptosis network, although each node of intervention carries its own risk and success profile. Given that ferroptosis is driven by phospholipid peroxidation, one strategy is to introduce agents that prevent the peroxidation process, for example, by preventing the propagation of lipid peroxyl radicals through the administration of lipophilic radical-trapping antioxidants, such as liproxstatin 1 (REF.[51]). These agents are highly effective in cell models of ferroptosis and can also be effective in some in vivo contexts. Their pharmacological properties need to be further improved before human trials can be considered. A related approach is to administer PUFAs that are chemically resistant to peroxidation, such as by incorporation of deuterium at the bis-allylic carbon that is normally susceptible to peroxidation[37,176]. Such deuterated PUFAs have been shown to be effective inhibitors of ferroptosis and various chronic ferroptosis-linked degenerative diseases, although with lower potency than ferrostatin 1 and liproxstatin 1 (REFS[37,103]). Similarly, supply of MUFAs has been shown to suppress ferroptosis and could be exploited as a potential therapy[36,37].

A second strategy for blocking lipid peroxidation is to either deplete the labile iron pool, for example through using iron chelators, or to inhibit enzymes that drive lipid peroxidation. Iron chelators have been explored in numerous clinical indications but have safety issues to contend with, due to potential off-target effect of these

Excitotoxic cell death
A form of neuronal cell death elicited by exposure of glutamate receptor-expressing neurons to excess glutamate or related excitatory amino acids.

agents and, more importantly, the essential biological function of iron. Targeting enzymes responsible for generating PUFA-PLs and PLOOHs, such as ACSL4, LPCAT3, LOXs and POR, might be feasible, and as defined molecular targets they are preferable from a target-centric drug discovery standpoint. However, they also have other functions in various tissues, complicating the potential therapeutic outcomes.

Conclusions and perspectives

Description of ferroptosis as a unique cell death modality brings together into a cohesive network previously disparate elements of cell metabolism, involving iron, selenium, amino acids, lipids and redox chemistry (FIG. 1). As the studies of ferroptosis advance, we are starting to acknowledge that this network functions in a broad array of biological processes, including both normal physiology and diverse pathologies. Below, we would like to share some concerns, challenges, and a preview of potential discoveries to be made in this exciting field.

First, we share some concerns. Although there is enthusiasm for the increasing appreciation of the interdigitation of ferroptosis with diverse aspects of biology, care must be taken to rigorously test the role of ferroptosis in biological processes. Suppression of cell death by a single inhibitor of ferroptosis cannot be taken as sufficient evidence that ferroptosis is involved in a process of interest. The current operational definition of

ferroptosis[177] is that of a cell death process suppressed by both iron depletion and lipophilic radical-trapping antioxidants, such as ferrostatin 1, liproxstatin 1, vitamin E or ubiquinone. Both requirements are critical, as on the one hand there are iron-dependent lethal mechanisms distinct from ferroptosis[178] that may involve, for example, lysosomal toxicity and, on the other, oxidative stress mechanisms independent of iron are well-established[1]. We suggest adding another requirement: direct detection of lipid peroxidation (using mass spectrometry, fluorescent dyes or antibodies such as 1F83). The mobilization and upregulation of transferrin receptor (TfR1) is another potential marker of ferroptosis recently reported[179,180], which allows for the differentiation of contexts involving oxidative stress versus ferroptosis. Still, additional markers of ferroptosis would be highly valuable to the field. Beyond definition, another significant concern in the field is the use of experimental approaches that are not suitable to address the intended question — an inevitable problem in any newly emerging area. To address these issues, BOX 2 and BOX 3 present some practical guidelines for ferroptosis investigation, in which important considerations for in vivo and in vitro study of ferroptosis are described.

Second, we note a major challenge. Despite considerable progress in understanding the mechanisms regulating ferroptosis, we still do not know what exact molecular event is responsible for eventual cell death via ferroptosis, that is, the point of no return. Uncontrolled peroxidation of PUFA-PLs is the most downstream step identified; it may be that peroxidized phospholipids cause membrane damage or even pore formation, compromising membrane integrity. However, a recent report found that phospholipids containing two PUFA tails are particularly effective drivers of ferroptosis[89], suggesting that lipid cross-linking may be an aspect of the membrane damage upon ferroptosis. In this scenario, it may be that limiting fluidity of membrane components by cross-linked lipids causes failure of some vital functions associated with membranes, resulting in cell death. However, other possibilities exist, such as that oxidized PUFA-PLs decompose into reactive electrophiles that then damage other macromolecules. In this model, reactive electrophiles serve the function in ferroptosis that caspases serve in apoptosis — to inactivate key structural and functional proteins within cells. We hope that over the next several years, the mechanism of ferroptosis execution will be unambiguously elucidated.

Last, we can predict some potential future discoveries that lie ahead in the field of ferroptosis. Identifying precise in vivo biomarkers for ferroptosis, a role cleaved caspase 3 serves for apoptosis, will be fundamentally important for determining the physiological function and therapeutic potential of this cell death modality. Identifying the enigmatic natural triggers or physiological signals that serve to trigger ferroptosis in non-pathological contexts also remains a pressing challenge, but one that may see breakthroughs in the coming years. Some molecules that might be considered as, or proximate to, the pathophysiological triggers of ferroptosis include glutamate, p53, iron, radiation and PUFA-PLs. Glutamate is an abundant neurotransmitter, and excess extracellular

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Box 2 | **Potential caveats to using models of ferroptosis induction**

Ferroptosis induction is typically modelled by interfering with the key components of the ferroptosis-suppressing pathways, in particular the cyst(e)ine–system x_c^- cystine/glutamate antiporter–glutathione (GSH)–glutathione peroxidase 4 (GPX4) axis. It needs to be considered that the outcome of these perturbations will depend on the cellular and environmental context in which they are introduced.

Sensitivity to ferroptosis is inevitably linked to intracellular and extracellular redox conditions. For instance, system x_c^- subunit *SLC7A11* expression is upregulated in many cell lines and promptly induced in ex vivo-cultured cells and organs due to the virtual absence of cysteine in culture media. Whereas knockout of *Slc7a11* is well tolerated in mice because cysteine is present in vivo in its reduced form that is taken up into cells by neutral amino acid transporters[146], almost all cell lines expressing system x_c^- that are subject to *SLC7A11* knockout invariably die when cultured in vitro as all cysteine is present in its oxidized form (as cystine) in culture medium. These stark discrepancies in redox conditions between cell culture-based and in vivo studies call for a careful interpretation of findings solely based on in vitro studies. Equally important, when using system x_c^- inhibitors, it needs to be assessed whether SLC7A11 is expressed at all in the cell line to be studied. Alarmingly, many currently available antibodies against SLC7A11 are non-specific and unsuitable for determining SLC7A11 levels. Moreover, as cell density has a profound impact on cells' sensitivity towards ferroptosis[17,41], this parameter needs to be carefully controlled in cell-based assays.

When interfering with GPX4 activity, one needs to consider that GPX4 is promiscuous in using as a substrate not only GSH but also other thiol-containing compounds and even protein thiols[26]. This is of particular significance when using ferroptosis-inducing agents that act at the different steps of the cyst(e)ine–system x_c^-–GSH–GPX4 axis. Notably, sera contain varying amounts of selenium as well as vitamin E. As GPX4 requires selenium for its biosynthesis and vitamin E is a potent antioxidant able to counteract phospholipid peroxidation, different cell culture sera may have a profound impact on the results. This is also of vital importance when using *Gpx4* knockout mice, as chows can vary in their vitamin E contents (both tocopherols and tocotrienols), which has a major impact on the phenotypes of *Gpx4* knockout mice[174,189,190]. Consistently, supplementation with selenium and vitamin E has been shown to be cancer-promoting in some contexts[191]. Therefore, such environmental variabilities should also be considered when investigating roles of ferroptosis.

> **Box 3 | Experimental consideration when using pharmacological tools to probe ferroptosis**
>
> Small-molecule tools are valuable for probing ferroptosis. However, several issues involving specificity, biological relevance, pharmacokinetics and pharmacodynamics must be considered when using such tools. First, when using pharmacological inhibitors of a certain enzyme to assess whether the enzyme regulates ferroptosis, one needs to determine whether the purported inhibitor can act as a lipophilic radical-trapping antioxidant and, thus, inhibit ferroptosis independently of the enzyme under study, as occurred in some studies involving inhibition of lipoxygenases[51,52,192]. Second, it is important to verify in the experimental system of interest whether a given small-molecule probe affects the intended target or pathway. For example, in using RSL3 to inhibit glutathione peroxidase 4 (GPX4) or erastin to inhibit the system x_c^- cystine/glutamate antiporter, it is important to test whether those targets are indeed inhibited by each probe in the experimental set-up using a biochemical assay or a pharmacodynamic marker of target inhibition. Moreover, when using small molecules in animal studies, it is important to first determine the appropriate formulation and route of administration to achieve an acceptable pharmacokinetic profile and pharmacodynamic response in the target tissue. Many small molecules that are potent and selective probes in cellular assays have no utility in animal models due to poor solubility, stability and/or pharmacokinetics. Some common approaches that are inappropriate for use in vivo, and thus should be avoided, include: RSL3, which has low solubility and its pharmacokinetics are difficult to measure in vivo, limiting its applicability to some cases involving direct injection into tissues or tumours; erastin, which also has low solubility and low metabolic stability (of note, its derivative imidazole ketone erastin (IKE) has good solubility, high stability and favourable pharmacokinetics and pharmacodynamics in mice, and has been validated for in vivo studies); and, similarly, ferrostatin 1 is not suitable for in vivo studies, whereas some other radical-trapping antioxidants such as improved ferrostatins, liproxstatin 1 and vitamin E can be used in vivo as ferroptosis inhibitors.
>
> There are also general considerations for interfering with iron as a means to modulate ferroptosis. Inducing or potentiating ferroptosis in cell culture by adding iron (ferric or ferrous) into the culture medium is not appropriate for the study of ferroptosis mechanisms, as free iron ions catalyse the Fenton chain reaction in the presence of oxygen and trace amounts of lipid peroxides (generated by accidental cell death or ferroptosis triggered by other inducers), thus amplifying extracellular lipid peroxidation and killing cells rapidly. In vivo data generated using iron chelators as a sole means to prevent ferroptosis should also be treated with caution, as depleting iron may lead to a plethora of consequences in addition to ferroptosis inhibition, and it is unclear whether a specific outcome is caused by iron chelation or an off-target activity of the compounds.

glutamate is sufficient to inhibit system x_c^-, resulting in ferroptosis[1]. Nature may exploit this effect of extracellular glutamate to induce ferroptosis in developmental or other physiological contexts, such as the elimination of unnecessary neurons in the sculpting of neural circuits. The tumour suppressor p53 induces ferroptosis to suppress tumour development; induction of p53 in the presence of basal lipid peroxidative damage may be a natural means of eliminating stressed cells through ferroptosis. High levels of iron alone can trigger ferroptosis in diverse model systems, suggesting that, in some contexts, releasing ferritin-stored iron or increasing iron import might be a means of eliminating cells through ferroptosis. Low to moderate doses of ionizing radiation have been shown to induce ferroptosis. Thus, contexts in which radiation is abundant or radioprotective cellular mechanisms are compromised might be circumstances in which ferroptosis is naturally induced. In this context, there is also speculation that there might once have been life on Mars, which either has been lost or has moved underground due to the harsh radiation present on the planet surface. Thus, it could be speculated that the ferroptotic programme is inherent to all life, and needs to be continuously counteracted — by establishing a network of anti-ferroptotic mechanisms — for life to survive and evolve; downregulating these protective mechanisms would then serve as a natural trigger for driving ferroptosis. Finally, PUFA uptake and PUFA-PL synthesis may be sufficient in some contexts to induce ferroptosis; in such cases, a cell could be induced to die by ferroptosis by the presence of PUFAs in the extracellular environment (for example, released by other cells or pathogens), or by being stimulated to drive expression of enzymes such as LPCAT3 and ACSL4 that promote PUFA-PL synthesis. Some of these mechanisms may be involved in development and regulation of tissue size, tumour suppression or clearance of infected cells by the immune system.

In summary, there is a wealth of foreseeable opportunities to elucidate both the execution mechanisms of ferroptosis and the contexts in which this form of cell death is naturally harnessed. Such studies will illuminate the breadth of physiological and pathological roles of ferroptosis. We also predict that novel ferroptosis-based therapies, guided by the use of specific biomarkers and precise evaluation of the pathogenetic background of patients, will be developed and put to use clinically in the near future.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

X.J. is an inventor on patents and patent applications involving programmed cell death and autophagy. B.R.S. is an inventor on patents and patent applications involving ferroptosis, co-founded and serves as a consultant to Inzen Therapeutics and Nevrox Limited, and serves as a consultant to Weatherwax Biotechnologies Corporation. M.C. is an inventor on patents for some of the compounds described herein, and co-founder of ROSCUE Therapeutics GmbH.

Peer review information

Nature Reviews Molecular Cell Biology thanks F. Wang, A. Bush and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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