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Targeting ferroptosis as a vulnerability in cancer

Guang Lei¹, Li Zhuang¹ and Boyi Gan^{1,2}✉

Abstract | Ferroptosis is an iron-dependent form of regulated cell death that is triggered by the toxic build-up of lipid peroxides on cellular membranes. In recent years, ferroptosis has garnered enormous interest in cancer research communities, partly because it is a unique cell death modality that is mechanistically and morphologically different from other forms of cell death, such as apoptosis, and therefore holds great potential for cancer therapy. In this Review, we summarize the current understanding of ferroptosis-inducing and ferroptosis defence mechanisms, dissect the roles and mechanisms of ferroptosis in tumour suppression and tumour immunity, conceptualize the diverse vulnerabilities of cancer cells to ferroptosis, and explore therapeutic strategies for targeting ferroptosis in cancer.

Cristae

Folds of the inner mitochondrial membrane that extend into the matrix of a mitochondrion.

Ferroptosis inducers (FINs). A compound or treatment that can induce ferroptosis by boosting ferroptosis-promoting mechanisms and/or suppressing ferroptosis defence mechanisms.

Ferroptosis, a term coined by Dixon et al. in 2012 (REF.¹), refers to an iron-dependent form of regulated cell death driven by an overload of lipid peroxides on cellular membranes. It is morphologically and mechanistically distinct from apoptosis and other types of regulated cell death; for example, morphologically, cells undergoing ferroptosis do not exhibit typical apoptotic features (such as chromatin condensation and apoptotic body formation) but are characterized by shrunken mitochondria and reduced numbers of mitochondrial cristae^{1,2}.

The lethal accumulation of lipid peroxides is a cardinal feature of ferroptosis³ and involves an antagonism between ferroptosis execution and ferroptosis defence systems in cells; ferroptosis occurs when ferroptosis-promoting cellular activities significantly override the antioxidant-buffering capabilities provided by ferroptosis defence systems^{4–10} (FIG. 1a). This mechanistic feature is markedly different from several other forms of regulated cell death that centre on cell death executioner proteins (such as caspase-mediated apoptosis, mixed lineage kinase domain-like protein (MLKL)-mediated necroptosis, and gasdermin D-mediated pyroptosis)¹¹. Finally, ferroptotic cells exhibit distinctive oxidized phospholipid (PL) profiles that differ from those of cells undergoing other forms of cell death^{12,13}.

Ferroptosis, as a unique cell death mechanism, has sparked great interest in the cancer research community as targeting ferroptosis might provide new therapeutic opportunities in treating cancers that are refractory to conventional therapies. In recent years, substantial progress has been achieved in understanding the role of ferroptosis in tumour biology and cancer therapy. On the one hand, multiple cancer-associated signalling pathways have been shown to govern ferroptosis in cancer cells¹⁴. The engagement of ferroptosis in the

activities of several tumour suppressors, such as p53 and BRCA1-associated protein 1 (BAP1), establishes ferroptosis as a natural barrier to cancer development^{15,16}, whereas oncogene-mediated or oncogenic signalling-mediated ferroptosis evasion contributes to tumour initiation, progression, metastasis and therapeutic resistance^{17–19}. On the other hand, the distinctive metabolism of cancer cells, their high load of reactive oxygen species (ROS) and their specific mutations render some of them intrinsically susceptible to ferroptosis, thereby exposing vulnerabilities that could be therapeutically targetable in certain cancer types^{20–24}. Furthermore, some cancer cells appear to be particularly dependent on ferroptosis defence systems to survive under metabolic and oxidative stress conditions; consequently, disruption of those defences would be fatal to such cancer cells while sparing normal cells⁹. These recent data suggest that ferroptosis represents a targetable vulnerability of cancer in certain contexts. Ferroptosis has also been recognized as a critical cell death response triggered by a variety of cancer therapies, including radiotherapy (RT), immunotherapy, chemotherapy and targeted therapies^{25–28}. Thus, ferroptosis inducers (FINs) hold great potential in cancer therapy (BOX 1), especially in combination with conventional therapies^{25,29,30}.

With the exponential growth in ferroptosis research in the past 4 years, iterative insights into how to target ferroptosis in cancer are critically needed. In this Review, we summarize the current understanding of the regulatory networks of ferroptosis, including the prerequisites for ferroptosis and ferroptosis defence systems, thoroughly analyse the mechanistic bases of ferroptosis in tumour biology, and synthesize a conceptual framework for how to target ferroptosis as a vulnerability in cancer therapy. Lastly, we highlight several key questions and challenges for future studies.

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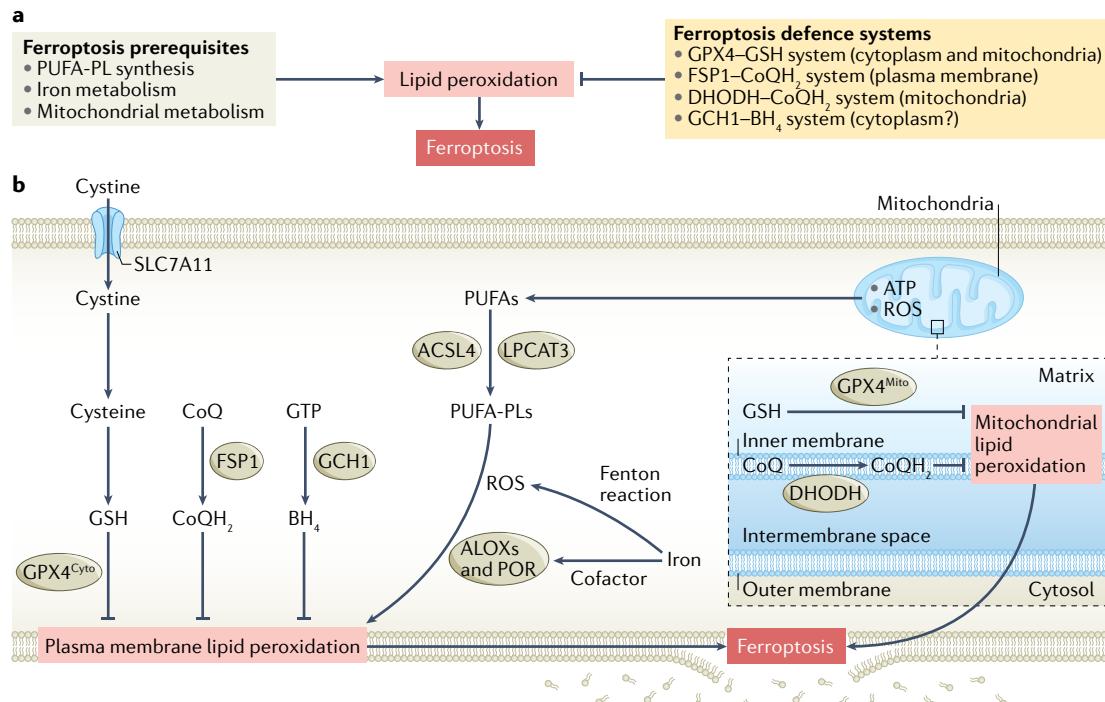


Fig. 1 | Ferroptosis-driving and ferroptosis defence mechanisms. **a** | Ferroptosis reflects an antagonism between prerequisites for ferroptosis and ferroptosis defence systems. The prerequisites for ferroptosis consist of polyunsaturated fatty acid-containing phospholipid (PUFA-PL) synthesis and peroxidation, iron metabolism, and mitochondrial metabolism. Ferroptosis defence systems mainly include the glutathione peroxidase 4 (GPX4)-reduced glutathione (GSH) system, the ferroptosis suppressor protein 1 (FSP1)-ubiquinol (CoQH₂) system, the dihydroorotate dehydrogenase (DHODH)-CoQH₂ system, and the GTP cyclohydrolase 1 (GCH1)-tetrahydrobiopterin (BH₄) system. When ferroptosis-promoting cellular activities significantly exceed the detoxification capabilities provided by ferroptosis defence systems, a lethal accumulation of lipid peroxides on cellular membranes lead to subsequent membrane rupture and ferroptotic cell death. **b** | Acyl-coenzyme A synthetase long chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) mediate the synthesis of PUFA-PLs, which are susceptible to peroxidation through both non-enzymatic and enzymatic mechanisms. Iron initiates the non-enzymatic Fenton reaction and acts as an essential cofactor for arachidonate lipoxygenases (ALOXs) and cytochrome P450 oxidoreductase (POR), which promote lipid peroxidation, and mitochondrial metabolism promotes the generation of reactive oxygen species (ROS), ATP and/or PUFA-PLs. Excessive accumulation of lipid peroxides on cellular membranes can trigger ferroptosis. Cells have evolved at least four defence systems with different subcellular localizations to detoxify lipid peroxides and thus protect cells against ferroptosis, wherein cytosolic GPX4 (GPX4^{Cyto}) cooperates with FSP1 on the plasma membrane (and other non-mitochondrial membranes) and mitochondrial GPX4 (GPX4^{Mito}) cooperates with DHODH in the mitochondria to neutralize lipid peroxides. The subcellular compartment in which the GCH1-BH₄ system operates remains to be defined. CoQ, coenzyme Q (also known as ubiquinone); SLC7A11, solute carrier family 7 member 11.

Ferroptosis prerequisites

The crux of ferroptosis execution is iron-catalysed peroxidation of polyunsaturated fatty acid (PUFA)-containing PLs (PUFA-PLs), which, when exceeding the buffering capability of ferroptosis defence systems (see the next section), can lead to lethal accumulation of lipid peroxides on cellular membranes and subsequent membrane rupture, resulting in ferroptotic cell death. As outlined in this section, PUFA-PL synthesis and peroxidation, iron metabolism, and mitochondrial metabolism constitute the main prerequisites driving ferroptosis^{3,31} (FIG. 1a).

PUFA-PL synthesis and peroxidation. Acyl-coenzyme A (CoA) synthetase long chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) are critical mediators of PUFA-PL synthesis^{3,32,33} (FIG. 1b). ACSL4 catalyses the ligation of free PUFAs, such as arachidonic acids and adrenic acids, with CoA to generate

PUFA-CoAs (such as arachidonic acid-CoA or adrenic acid-CoA)^{32,33}, which are subsequently re-esterified and incorporated into PLs by LPCAT3 to form PUFA-PLs (such as arachidonic acid-phosphatidylethanolamine or adrenic acid-phosphatidylethanolamine)^{13,32}. Acetyl-CoA carboxylase (ACC)-catalysed carboxylation of acetyl-CoA generates malonyl-CoA, which is required for the synthesis of some PUFAs (likely at the elongation steps) and therefore for ferroptosis^{32,34–36}. Inactivation of ACSL4, LPCAT3 or ACC blocks or attenuates ferroptosis^{13,33,37}. Interestingly, p53-mediated ferroptosis, in some contexts, seems to be independent of ACSL4 (REF.³⁸), although how ferroptosis occurs in the absence of ACSL4-mediated PUFA-PL synthesis remains unclear. Recent findings also revealed that PUFA-containing ether PLs (PUFA-ePLs) synthesized in peroxisomes act as additional substrates for lipid peroxidation, favouring ferroptosis onset^{23,39,40}.

Polyunsaturated fatty acid (PUFA). A fatty acid that contains more than one double bond and is required for cellular signalling and membrane fluidity.

Peroxisomes
Organelles that are important for β -oxidation of very-long-chain fatty acids and synthesis of ether phospholipids.

Fenton reaction

A non-enzymatic reaction of labile iron and H₂O₂ that generates hydroxide and hydroxyl radicals, which can subsequently induce lipid peroxidation.

Anaplerotic reactions

Metabolic reactions that replenish the supply of intermediates involved in the citric acid cycle.

PUFA-PLs are particularly susceptible to peroxidation due to the presence of bis-allylic moieties in PUFAs⁴¹. The peroxidation of PUFA-PLs is primarily catalysed by non-enzymatic autoxidation driven by the Fenton reaction with iron as a catalyst^{41–43} (FIG. 1b). Cytochrome P450 oxidoreductase (POR)-mediated or arachidonate lipoxygenase (ALOX)-mediated enzymatic reactions have also been shown to promote lipid peroxidation^{44–48}; although the ability of POR to promote lipid peroxidation appears to be indirect via the generation of H₂O₂ (REF. ⁴⁶) and the role of ALOXs in ferroptosis has been challenged by other studies, including the lack of rescue of glutathione peroxidase 4 (*Gpx4*; part of a ferroptosis defence mechanism) knockout phenotypes by *Alox15* deletion in mice⁴⁹. Monounsaturated fatty acids (MUFA), such as oleic acid and palmitoleic acid, are not readily peroxidized owing to their lack of bis-allylic moieties⁵⁰. In contrast to PUFAs, MUFA restrain lipid peroxidation and ferroptosis by displacing PUFAs from PLs in cellular membranes; inactivation of enzymes involved in MUFA-PL synthesis, such as stearoyl CoA desaturase 1 (SCD1) and ACSL3, sensitizes cancer cells to ferroptosis^{50–52}.

Iron metabolism. Iron governs ferroptosis not only by initiating the non-enzymatic Fenton reaction for direct peroxidation of PUFA-PLs^{41,43} but also by acting as an

Box 1 | Ferroptosis inducers

Ferroptosis inducers (FINs) are compounds or treatments that induce ferroptosis in cells and can be categorized into at least four classes based on their mechanisms of action¹⁷⁰. Class I FINs act by inhibiting solute carrier family 7 member 11 (SLC7A11)-mediated cystine uptake and restricting intracellular cysteine and glutathione levels^{82,170}. Erastin is the most widely used class I FIN in cell culture studies, although its application *in vivo* is limited by its poor metabolic stability and water solubility¹⁷⁰. Its analogue imidazole ketone erastin has improved potency, solubility, and metabolic stability¹⁷¹ and has been shown to induce ferroptosis and suppress tumour growth *in vivo*²⁹. Sulfasalazine, an FDA-approved anti-inflammatory agent with class I FIN activity^{172,173}, can inhibit tumour growth in diverse preclinical models^{97,173}. Although sulfasalazine exhibits relatively poor metabolic stability and potency¹⁷⁰, its activity in patients with glioblastoma has been demonstrated¹⁷⁴ and its combination with radiotherapy is being tested in a clinical trial for glioblastoma (NCT04205357)¹⁶¹. Sorafenib, another clinically approved agent, is a multi-kinase inhibitor with activity against SLC7A11 (REF. ¹⁴⁴); although it has been shown to induce ferroptosis, it also induces other non-ferroptotic cellular effects^{144,170}, and its classification as a FIN was challenged by a recent study¹⁷⁵. In addition, cyst(e)inase degrades extracellular cystine and cysteine and has been shown to act as a potent and tolerable FIN to suppress tumour growth *in vivo* when used either as a treatment alone or in combination with immune-checkpoint inhibitors^{28,176}.

Class II FINs operate by blocking the enzymatic activity of glutathione peroxidase 4 (GPX4), and include RSL3, ML162 and ML210. RSL3 and ML162 directly inhibit the catalytic activity of GPX4 by covalently binding to the selenocysteine residue of GPX4 through their electrophile chloroacetamide moiety^{4,165}; however, their application *in vivo* is limited by their low solubility and poor pharmacokinetics^{165,170}. ML210 is a nitroisoxazole-containing compound that is converted to α-nitroketoimine (known as JKE-1674) in cells and subsequently forms a nitrile-oxide electrophile, which covalently inhibits GPX4 with remarkable selectivity¹⁶⁵. Notably, JKE-1674 exhibits improved pharmacokinetic properties¹⁶⁵, although its effect on tumour growth remains to be investigated. Moreover, altretamine (an FDA-approved anticancer agent) and withaferin A (a natural anticancer agent suitable for *in vivo* treatment) have been shown to inhibit GPX4 activity or decrease GPX4 levels, providing an alternative for targeting GPX4 *in vivo*^{166,167}.

Finally, class III FINs, such as FIN56 (REF. ³⁶), act by depleting both GPX4 protein and ubiquinone. Class IV FINs, such as FINO₂ (REF. ¹⁷⁷), act by oxidizing iron and indirectly inactivating GPX4. However, these FINs have not been assessed *in vivo*.

essential cofactor for enzymes that participate in lipid peroxidation (such as ALOX and POR)^{44,45}. Cells normally maintain a relatively stable pool of labile iron through orchestrated regulation of iron uptake, utilization, storage and export⁵³. Imbalanced regulation of these iron metabolism processes can promote or suppress ferroptosis, depending on whether the level of the intracellular labile iron pool is increased or decreased, respectively. For example, intracellular iron is stored mostly as inert iron in ferritin, whereas autophagic degradation of ferritin (ferritinophagy) releases iron stored in ferritin into the labile iron pool; consequently, blockade of nuclear receptor coactivator 4 (NCOA4)-mediated ferritinophagy decreases the level of the labile iron pool and suppresses ferroptosis^{54,55}. Conversely, enhancement of ferritinophagy by inhibition of cytosolic glutamate oxaloacetate transaminase 1 (GOT1) increases the labile iron pool and promotes ferroptosis (although exactly how GOT1 inhibition promotes ferritinophagy-mediated labile iron release remains to be further studied)⁵⁶. We refer readers to a recent excellent review for detailed discussion of iron metabolism in ferroptosis⁵³.

Mitochondrial metabolism. Several metabolic processes in mitochondria have important roles in triggering ferroptosis³¹ (FIG. 1b). First, the generation of mitochondrial ROS is critical for lipid peroxidation and ferroptosis onset. Mitochondria are a major source of cellular ROS, in which electron leakage from electron transport chain complexes I and III generates superoxides, followed by their conversion to H₂O₂ by superoxide dismutase⁵⁷. H₂O₂ can then react with labile iron via the Fenton reaction to generate hydroxyl radicals, which subsequently drive PUFA-PL peroxidation^{57,58}. Moreover, electron transport and proton pumping in mitochondria are important for ATP production^{59,60}, which also promotes ferroptosis^{35,37}. Specifically, under ATP-depleted conditions, AMP-activated protein kinase (AMPK) phosphorylates and inactivates ACC, thereby suppressing PUFA-PL synthesis and blocking ferroptosis; in contrast, with sufficient energy (that is, ATP), AMPK cannot be activated efficiently and ACC is activated, thus promoting PUFA-PL synthesis and ferroptosis^{35,37}. Finally, the role of mitochondria in biosynthetic pathways in cellular metabolism also contributes to ferroptosis. Ferroptosis requires the tricarboxylic acid (TCA) cycle and various anaplerotic reactions that fuel it (such as glutaminolysis) in mitochondria⁵⁹, which drive ferroptosis likely through the promotion of ROS, ATP and/or PUFA-PL generation^{61–63}. Therefore, the multifaceted functions of mitochondria in bioenergetics, biosynthesis and ROS generation drive mitochondrial lipid peroxidation and ferroptosis³¹.

Ferroptosis defence mechanisms

Ferroptosis defence mechanisms involve cellular antioxidant systems that directly neutralize lipid peroxides. The study of ferroptosis defence mechanisms has been one of the most exciting and rapidly evolving areas in ferroptosis research in the past 2 years. As discussed below, there are at least four such ferroptosis defence systems with distinctive subcellular localizations (FIG. 1).

GPX

A family of peroxidases that use reduced glutathione as their cofactor to reduce hydroperoxide species to their corresponding alcohols.

System xc⁻

An antiporter that imports cystine and exports glutamate; it consists of two subunits, including the transporter subunit SLC7A11 and the regulatory subunit SLC3A2.

Ubiquinone

Also known as coenzyme Q or CoQ. A lipophilic molecule that is composed of a quinone head group linked to a polyisoprenoid lipid tail and acts as an electron transport carrier in mitochondria.

Ubiquinol

(CoQH₂). The fully reduced form of ubiquinone.

Dihydroorotate dehydrogenase (DHODH)

An inner mitochondrial membrane-localized enzyme that oxidizes dihydroorotate to orotate for pyrimidine synthesis while reducing CoQ to CoQH₂.

The GPX4–GSH system. GPX4 belongs to the GPX protein family^{64,65} and is the only GPX member capable of converting PL hydroperoxides to PL alcohols^{66,67}. Genetic ablation or pharmacological inhibition of GPX4 induces unchecked lipid peroxidation and triggers potent ferroptosis under many *in vitro* and *in vivo* conditions^{4,49,66}. GPX4 consists of three isoforms with distinctive subcellular localizations, namely cytosolic, mitochondrial and nuclear GPX4. These isoforms are encoded by the same *GPX4* gene with different transcription initiation sites, resulting in the addition of a mitochondrial or nuclear localization sequence to the N-terminus of the GPX4 protein^{68–71}. Until very recently, it was believed that only cytosolic GPX4 had a role in defending against ferroptosis because it is required for embryonic development whereas the mitochondrial or nuclear isoforms are not^{72–75} and because re-expression of cytosolic GPX4 but not of mitochondrial or nuclear GPX4 significantly suppresses cell death induced by *Gpx4* deletion in mouse embryonic fibroblasts⁷⁶. However, recent studies suggest that both cytosolic and mitochondrial GPX4 are important in defending against ferroptosis in different subcellular compartments (see below)⁹. The potential role of nuclear GPX4 in the regulation of ferroptosis remains to be studied.

Reduced glutathione (GSH), the cofactor used by GPX4, is a thiol-containing tripeptide derived from glycine, glutamate and cysteine, with cysteine being the rate-limiting precursor^{77,78}. Most cancer cells obtain intracellular cysteine primarily through system xc⁻-mediated uptake of cystine (an oxidized dimeric form of cysteine), followed by cystine reduction to cysteine in the cytosol^{79,80}. Solute carrier family 7 member 11 (SLC7A11; also known as xCT) is the transporter subunit in system xc⁻ (REFS^{81,82}). Removing cystine from culture media or pharmacologically blocking SLC7A11-mediated cystine transport with erastin or other FINs induces potent ferroptosis in many cancer cell lines^{1,15,16,82}. The SLC7A11–GSH–GPX4 axis is believed to constitute the major cellular system defending against ferroptosis (FIG. 1b). However, upon GPX4 inactivation, some cancer cell lines remain resistant to ferroptosis²², suggesting the existence of additional ferroptosis defence mechanisms.

The FSP1–CoQH₂ system. While it was initially believed that GPX4 was the only ferroptosis defence system, this paradigm shifted following recent studies revealing that ferroptosis suppressor protein 1 (FSP1; also known as AIFM2) operates independently of GPX4 to defend against ferroptosis^{5,6}. FSP1 localizes on the plasma membrane (as well as on other subcellular compartments) and its plasma membrane localization appears to be both necessary and sufficient for the function of FSP1 in suppressing ferroptosis^{5,6}. FSP1 functions as an NAD(P)H-dependent oxidoreductase capable of reducing ubiquinone (also known as coenzyme Q or CoQ)^{83,84} to ubiquinol (CoQH₂)^{5,6} (FIG. 1b). Apart from its well-known function in mitochondrial electron transport, CoQH₂ can also trap lipid peroxy radicals, thereby suppressing lipid peroxidation and ferroptosis. Therefore, it has been proposed that FSP1 exerts its potent antiferroptosis activity through the generation of

the non-mitochondrial CoQH₂ pool as radical-trapping antioxidants^{5,6}. It should be noted that CoQ is mainly synthesized in mitochondria⁸³ but has been detected in non-mitochondrial membranes, including the plasma membrane^{85–88}. The sources of the non-mitochondrial CoQ utilized by FSP1 for ferroptosis defence remain to be established.

The DHODH–CoQH₂ system. A recent study uncovered a mitochondria-localized defence system mediated by dihydroorotate dehydrogenase (DHODH) that can compensate for GPX4 loss to detoxify mitochondrial lipid peroxidation⁹. DHODH is an enzyme involved in pyrimidine synthesis that can reduce CoQ to CoQH₂ in the inner mitochondrial membrane (FIG. 1b). When GPX4 is acutely inactivated, the flux through DHODH is significantly increased, resulting in enhanced CoQH₂ generation that neutralizes lipid peroxidation and defends against ferroptosis in mitochondria⁹. Consequently, inactivation of both mitochondrial GPX4 and DHODH unleashes potent mitochondrial lipid peroxidation and triggers robust ferroptosis. Importantly, while mitochondrial GPX4 and DHODH can compensate for each other to suppress mitochondrial lipid peroxidation, cytosolic GPX4 and FSP1 fail to do so⁹, ostensibly because they are not localized in mitochondria and therefore cannot detoxify lipid peroxides accumulated in the inner mitochondrial membrane, highlighting the importance of compartmentalization in ferroptosis defence.

These recent studies suggest a model whereby ferroptosis defence systems can be divided into two major divisions, the GPX4 system and the CoQH₂ system, with each of these further subdivided into arms located in non-mitochondrial and mitochondrial compartments (that is, cytosolic and mitochondrial GPX4 in the GPX4 system, versus non-mitochondrial FSP1 and mitochondrial DHODH in the CoQH₂ system). The rationale for this division in ferroptosis defence is likely derived from the significant need to mitigate lipid peroxides generated in mitochondria (see subsection on mitochondrial metabolism) and the double-membrane structure of mitochondria (which prohibits cytosol-localized or other compartment-localized defence systems from entering the mitochondria). Further research is required to substantiate this compartmentalization model in ferroptosis regulation and to reconcile with some conflicting data from previous studies. For example, cytosolic GPX4 has also been found to significantly localize in the intermembrane space of mitochondria⁸⁴. The abundance of cytosolic GPX4 in mitochondria and its potential role in suppressing lipid peroxidation within mitochondria remain to be clarified by future investigations.

Another open question concerns potential roles of other mitochondrial enzymes that produce CoQH₂, most notably electron transport chain complexes I and II, in ferroptosis regulation. However, unlike DHODH inactivation, inactivation of complex I or II by pharmacological inhibition does not appear to affect GPX4 inactivation-induced ferroptosis, and its inactivation even promotes resistance to cystine starvation-induced ferroptosis⁶². Conceivably, the presumed antiferroptosis function of complex I or II

Hypercholesterolaemia
High levels of cholesterol in the blood.

Lipid droplets
Organelles with a phospholipid monolayer that are responsible for lipid storage, including PUFA storage.

Iron–sulfur clusters
(ISCs). Molecular ensembles of iron and sulfur that function as protein co-factors to regulate iron homeostasis and redox reactions in response to oxidative stress.

(by producing mitochondrial CoQH₂) might be offset by its pro-ferroptosis functions (through the production of ROS or ATP; see subsection on mitochondrial metabolism). The potential role of additional CoQH₂-producing enzymes localized in mitochondria, such as electron transfer flavoprotein dehydrogenase, in ferroptosis regulation awaits further research.

The GCH1–BH₄ system. Recent studies identified GTP cyclohydrolase 1 (GCH1) as another critical regulator of ferroptosis^{7,8}. Tetrahydrobiopterin (BH₄) is a cofactor of aromatic amino acid hydroxylases and other enzymes, and GCH1 mediates the rate-limiting reaction in the BH₄ biosynthesis pathway⁸⁹. BH₄ is another radical-trapping antioxidant capable of trapping lipid peroxy radicals, and its function in inhibiting ferroptosis appears to be independent of its role as a cofactor⁸. It was proposed that GCH1 suppresses ferroptosis through the generation of BH₄ as a radical-trapping antioxidant as well as via GCH1-mediated production of CoQH₂ and PLs containing two PUFA tails^{7,8} (whereas most PUFA-PLs tend to exhibit an asymmetric structure, with a PUFA tail at the *sn*-2 position and a saturated fatty acid tail at the *sn*-1 position) (FIG. 1b). The subcellular compartment wherein the GCH1–BH₄ system operates remains to be defined.

Box 2 | Ferroptosis regulation by tumour suppressors

p53, the most frequently mutated tumour suppressor¹⁷⁸, has an important role in regulating ferroptosis^{15,179}. p53 inhibits solute carrier family 7 member 11 (SLC7A11) expression through binding directly to the SLC7A11 promoter or via interacting with ubiquitin-specific processing protease 7 (USP7) to reduce histone H2B monoubiquitination levels on the SLC7A11 promoter, sensitizing cancer cells to ferroptosis in an arachidonate 12-lipoxygenase (ALOX12)-dependent manner^{15,38,142,180}. p53 can also promote ferroptosis by modulating additional metabolic targets^{181–183}. Importantly, some p53 mutants cannot induce apoptosis, senescence or cell cycle arrest, yet can promote ferroptosis to mediate tumour suppression *in vivo*¹⁵. In contrast, other p53 mutants lacking ferroptosis regulatory activity lose their tumour-suppression function^{183,184}. Note that p53 appears to play a dual role in ferroptosis regulation depending on the cellular context, as it can exert an antiferroptosis effect under certain conditions^{185,186}.

The tumour suppressor BAP1 encodes a nuclear deubiquitinating enzyme (DUB) that reduces histone H2A ubiquitination (H2Aub) on chromatin^{187,188}. BAP1 is frequently mutated in several sporadic cancers, including uveal melanoma, mesothelioma and renal cell carcinoma¹⁸⁹. BAP1 reduces H2Aub occupancy on the SLC7A11 promoter and represses SLC7A11 expression in a DUB-dependent manner, thereby inhibiting cystine uptake and promoting ferroptosis, which partly mediates the tumour-suppressive effect of BAP1 in xenograft models¹⁶. In addition, the ability of BAP1 to restrain SLC7A11 expression and promote ferroptosis is compromised in cells with cancer-associated BAP1 mutations¹⁶.

Kelch-like ECH associated protein 1 (KEAP1) is a tumour suppressor frequently mutated in non-small-cell lung cancer^{190,191}. It encodes a substrate adaptor that targets the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) for proteasomal degradation^{192,193}. KEAP1 inactivation in cancer leads to NRF2 protein stabilization^{192,193}. As a master regulator of antioxidant defence, NRF2 promotes the transcription of many genes governing ferroptosis suppression such as SLC7A11 (REFS^{99,101,194}). In addition, ARF is a well-established tumour suppressor that is critical for p53 activation in response to oncogenic stress¹⁹⁵. Interestingly, ARF was recently shown to inhibit NRF2 transcriptional activity independently of KEAP1 or p53 (REF.¹⁰⁰). In ARF-mutated tumours, NRF2 and its downstream target genes (such as SLC7A11) are activated, enabling cancer cells to evade ferroptosis under oxidative stress¹⁰⁰. Therefore, ferroptosis activation by KEAP1 or ARF due to NRF2 suppression is likely a tumour-suppressive mechanism.

Most studies have focused on ferroptosis regulation mediated by tumour suppressors through SLC7A11. The control of ferroptosis by other tumour suppressors through other mechanisms remains to be explored.

Ferroptosis evasion fuels tumours

Accumulating evidence indicates that ferroptosis is a critical tumour suppression mechanism. Several tumour-suppressor and oncogenic signalling pathways have been shown to promote or suppress ferroptosis, respectively (BOXES 2,3). Tumours have evolved at least three mechanisms to evade ferroptosis and to facilitate tumour development and metastasis, including limiting PUFA-PL synthesis and peroxidation, restricting labile iron availability, and upregulating cellular defence systems against ferroptosis.

Suppression of PUFA-PL synthesis and peroxidation.

Downregulation of peroxidized PUFA-PL levels in cancer cells has been linked to ferroptosis evasion and enhanced tumour growth. Calcium-independent phospholipase A₂β (iPLA₂β), a member of the iPLA₂ family, is overexpressed in some human cancers. iPLA₂β was recently shown to facilitate cancer cell escape from ferroptosis by hydrolysing peroxidized PLs, and iPLA₂β depletion sensitized cancer cells to ferroptosis and impaired xenograft tumour growth^{90,91}. Another recent study revealed that the expression of the adipokine chemerin is frequently upregulated in renal cell carcinoma (RCC), and that chemerin may maintain xenograft RCC growth through the downregulation of the levels of peroxidized PUFA-PLs and by promoting ferroptosis evasion⁹².

Recent findings highlight that ferroptosis evasion through the modulation of fatty acid metabolism also contributes to cancer metastasis. Melanoma generally undergoes regional metastasis through the lymphatic system prior to systemic metastasis through the blood. The lymphatic environment has been observed to promote the escape of cancer cells from oxidative stress and ferroptosis *in vivo*, partly due to abundant levels of oleic acid (a MUFA) and GSH as well as low levels of free iron in the lymphatic fluid; oleic acid protects melanoma cells from ferroptosis in an ACSL3-dependent manner¹⁸ (as discussed above, ACSL3-mediated MUFA-PL synthesis suppresses ferroptosis by displacing PUFAs from PLs). Importantly, ferroptosis resistance conferred by the lymphatic environment on cancer cells contributes to their subsequent survival during metastasis through the blood¹⁸. Likewise, under conditions of hypercholesterolaemia, cancer cells exhibit superior metastatic capabilities *in vivo*, which may be attributable to ferroptosis resistance caused by the increased accumulation of lipid droplets and MUFA in these cells⁹³.

Restriction of the labile iron pool. As an important cofactor in redox maintenance and iron homeostasis, iron–sulfur clusters (ISCs) and the associated regulatory pathways contribute to ferroptosis evasion in cancer cells by diminishing the labile iron pool⁹⁴. The cysteine desulfurase NFS1 is involved in ISC biosynthesis by harvesting sulfur from cysteine. NFS1 was found to be overexpressed in lung cancers and to be required for lung tumour growth *in vivo*, likely due to its ability to limit labile iron levels and protect cancer cells from ferroptosis⁹⁵. Frataxin, another protein involved in ISC

Mevalonate pathway
A metabolic pathway that synthesizes cholesterol, CoQ and steroid hormones.

Epithelial-to-mesenchymal transition (EMT). A process by which epithelial cells gradually lose their cell polarity and intercellular adhesion properties and acquire mesenchymal-like phenotypes, including migratory and invasive properties.

assembly, is upregulated in different cancer types and was shown to promote cancer cell resistance to ferroptosis and, thus, xenograft tumour growth⁹⁶. Likewise, another recent study revealed that CDGSH iron–sulfur domain-containing protein 2 (CISD2), a component of the iron–sulfur protein family that is highly expressed in head and neck cancers, enhances the ability of cancer cells to counteract ferroptosis in vitro, likely by modulating ISC and decreasing free iron levels⁹⁷. In addition, when breast cancer cells detach from the extracellular matrix, they can upregulate prominin 2 expression to promote the formation of ferritin-containing multi-vesicular bodies that export iron out of cells, thereby facilitating ferroptosis evasion in vitro⁹⁸. In some of the examples discussed above, whether ferroptosis evasion in cancer cells indeed contributes to enhanced tumour growth or metastasis in vivo remains to be established.

Upregulation of ferroptosis defences. Most current studies of these mechanisms have centred on SLC7A11, which is overexpressed in multiple human cancer types^{79,82}. As discussed in BOXES 2 and 3, inactivation of tumour suppressors such as p53, BAP1, Kelch-like ECH associated protein 1 (KEAP1) and ARF (p14), or activation

Box 3 | Ferroptosis regulation by oncogenes

In line with the concept that ferroptosis is a natural tumour-suppressor mechanism, several oncogene-driven cancers have evolved mechanisms to escape from ferroptosis.

The PI3K oncogenic signalling pathway is one of the most frequently mutated pathways in human cancers^{196,197}. It was recently demonstrated that cancer cells carrying PIK3CA activating mutations or PTEN deletion are generally resistant to ferroptosis¹⁷. Oncogenic activation of the PI3K signalling pathway promotes the activation of mTOR complex 1 (mTORC1)¹⁹⁷, which inhibits ferroptosis through at least three parallel mechanisms¹⁹⁸: promoting glutathione peroxidase 4 (GPX4) protein synthesis¹²⁰, suppressing autophagy-dependent ferroptosis¹⁹⁹ and boosting monounsaturated fatty acid-containing phospholipid (MUFA-PL) synthesis by increasing stearoyl coenzyme A desaturase 1 (SCD1) expression¹⁷. Thus, aberrant activation of mTORC1 allows cancer cells to evade ferroptosis, which might partly account for the oncogenic activity caused by mutations in the PI3K pathway.

The RAS family of proto-oncogenes are frequently mutated in human cancers^{200,201}. Notably, RAS-mutant cancer cells are characterized by high levels of intracellular cysteine and glutathione in response to oxidative stress via mechanisms involving solute carrier family 7 member 11 (SLC7A11) upregulation¹⁰³. Furthermore, pharmacological or genetic inhibition of SLC7A11 severely impairs mutant KRAS-induced tumour growth, suggesting that SLC7A11 is required for mutant KRAS-mediated tumorigenesis^{102,103}. Therefore, SLC7A11-mediated cystine uptake and glutathione biosynthesis allow mutant RAS-driven tumours to evade ferroptosis under oxidative stress. It is also worth mentioning that KRAS mutation in lung cancer also increases acyl-coenzyme A synthetase long chain family member 3 (ACSL3) expression to reprogramme lipid metabolism, promoting MUFA-PL biosynthesis and ferroptosis resistance, and that these processes might be important for the development of mutant KRAS-driven lung cancer^{50,202}.

Several non-canonical oncogenes also contribute to the escape of cancer cells from ferroptosis. NEDD4, a ubiquitin ligase with oncogenic functions²⁰³, can be induced by the ferroptosis inducer erastin¹⁶⁹. Erastin-induced ferroptosis requires VDAC2/3 (REF.²⁰⁴); interestingly, VDAC2/3 expression was reduced in a NEDD4-dependent manner upon erastin treatment, thereby restricting erastin-induced ferroptosis¹⁶⁹. Other proteins with oncogenic activities, such as DJ1 (REF.²⁰⁵) and pyruvate dehydrogenase kinase 4 (PDK4)²⁰⁶, can also promote ferroptosis evasion through corresponding mechanisms^{145,149} (see section on targeting ferroptosis in cancer therapy). In all of these studies, the inactivation of NEDD4, DJ1 or PDK4 promoted ferroptosis and enhanced the tumour-suppressive effects of ferroptosis inducers in xenograft models^{145,149,169}. Whether ferroptosis evasion supports the oncogenic roles of these proteins remains to be further explored.

of oncogenic KRAS, upregulates SLC7A11 expression, which can be dependent or independent of nuclear factor erythroid 2-related factor 2 (NRF2), conferring ferroptosis evasion and promoting tumour growth^{15,16,99–103}. As a master regulator of antioxidant defence, the transcription factor NRF2 governs the transcription of many genes involved in GPX4-GSH-mediated ferroptosis defence, including SLC7A11, thereby facilitating the escape of cancer cells from ferroptosis. Consequently, NRF2 signalling is upregulated in many human cancer types^{99,101,104}. Interestingly, a recent study showed that treatment with 27-hydroxycholesterol (27-HC) leads to downregulation of GPX4 levels in breast cancer cells and that this effect is lost in breast cancer cells resistant to 27-HC, which likely endows such 27-HC-resistant breast cancer cells with increased resistance to ferroptosis and enhanced metastatic capability in vivo⁹³.

It appears that tumours also exploit other ferroptosis defence mechanisms. For example, GCH1 is overexpressed in multiple cancer types, including breast, lung and liver cancers, and its high expression correlates with ferroptosis resistance in vitro⁷. CoQH₂ used in ferroptosis defence is derived from the mevalonate pathway⁸³. Squalene monooxygenase (SQLE) is a key enzyme in the mevalonate pathway. A lack of SQLE expression in anaplastic lymphoma kinase (ALK)⁺ anaplastic large-cell lymphoma cells prevents cholesterol synthesis (thereby rendering such cancer cells cholesterol auxotrophic) and leads to the accumulation of the upstream metabolite squalene, a lipophilic antioxidant that protects cancer cells from ferroptosis in vivo¹⁰⁵. This mechanism provides a unique advantage for ALK⁺ anaplastic large-cell lymphoma growth under conditions of oxidative stress¹⁰⁵.

Ferroptosis vulnerability

Oncogenic mutations rewire cellular metabolic networks in cancer cells to fulfil their increased demand for nutrients and energy^{106,107}; this reprogramming often exposes new metabolic liabilities in cancer cells, rendering some of them uniquely vulnerable to ferroptosis. In this section, we conceptualize three such ferroptosis-based vulnerabilities in cancer cells, namely those induced by metabolic reprogramming, genetic mutations and an imbalance in ferroptosis defences (FIG. 2).

Metabolic features of cancer cells. Several studies revealed that therapy-refractory cancer cells in specific cellular states are unexpectedly sensitive to ferroptosis^{21,22,108}. For example, cancer cells in a mesenchymal state, which are usually resistant to apoptosis induced by conventional therapies, are strongly dependent on GPX4, and this dependency is associated with a high expression of zinc finger E-box binding homeobox 1 (ZEB1), a driver of epithelial-to-mesenchymal transition (EMT) and lipogenic factor¹⁰⁹. PUFA-PL synthesis is enhanced in cancer cells in the mesenchymal state, possibly owing to the central role of ZEB1 in lipid metabolism (FIG. 2a). The increased PUFA-PL levels in such cancer cells render them dependent on GPX4 to detoxify lipid peroxides for survival; consequently, these cells are highly vulnerable to ferroptosis²². Consistently, certain recurrent breast cancer cells with mesenchymal features exhibit

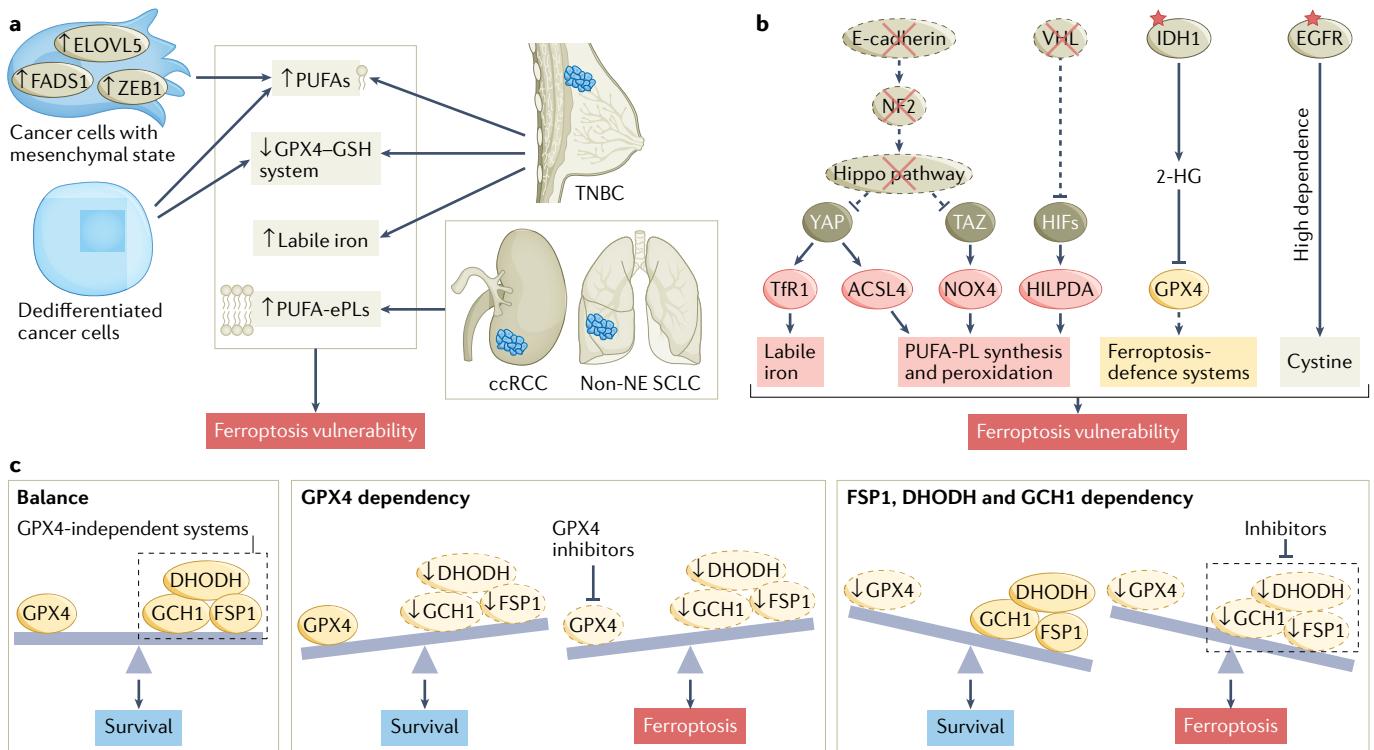


Fig. 2 | Ferroptosis as a vulnerability in cancer. **a** | Therapy-resistant cancer cells with specific cellular states are vulnerable to ferroptosis, for example, cancer cells with a mesenchymal phenotype that are enriched in polyunsaturated fatty acids (PUFAs) owing to high expression of zinc finger E-box binding homeobox 1 (ZEB1), elongation of very long-chain fatty acid protein 5 (ELOVL5) or fatty acid desaturase 1 (FADS1). Similarly, dedifferentiated subtypes of melanoma cells are characterized by PUFA accumulation and a deficiency of reduced glutathione (GSH), which render these cells vulnerable to ferroptosis. In addition, certain cancer cells, such as clear-cell renal cell carcinoma (ccRCC), non-neuroendocrine (NE) small-cell lung cancer (SCLC), and triple-negative breast cancer (TNBC) cells are inherently susceptible to ferroptosis owing to their unique metabolic features such as high levels of PUFA-containing ether phospholipids (ePLs). **b** | Mutations in certain tumour suppressors or oncogenes render cancers vulnerable to ferroptosis. Inactivating mutations in any constituent of the tumour-suppressive E-cadherin–neurofibromin 2 (NF2)–Hippo pathway confers a vulnerability to ferroptosis by upregulating Yes-associated protein (YAP)-mediated or transcriptional coactivator with PDZ-binding motif (TAZ)-mediated transcription of ferroptosis-promoting factors, such as acyl-coenzyme A synthetase long chain family member 4 (ACSL4), transferrin receptor 1 (TFR1) and NADPH oxidase 4 (NOX4). In ccRCC,

mutation or loss of *VHL* promotes hypoxia-inducible factor (HIF)-dependent expression of hypoxia-inducible, lipid droplet-associated protein (HILPDA), rendering ccRCCs vulnerable to ferroptosis. Non-small-cell lung cancers with epidermal growth factor receptor (EGFR) mutations are vulnerable to ferroptosis because of their high dependence on cystine. In another example, isocitrate dehydrogenase 1 (*IDH1*)-mutated cancer cells with increased levels of the oncometabolite 2-hydroxyglutarate (2-HG) are sensitive to ferroptosis owing to their decreased glutathione peroxidase 4 (GPX4) levels. Stars denote activating mutations in the corresponding proto-oncogenes, whereas red crosses denote inactivating mutation in or gene loss of corresponding tumour suppressors. **c** | Vulnerability to ferroptosis is triggered by an imbalance between GPX4-dependent and GPX4-independent ferroptosis defence systems. Cancer cells with low expression of components of GPX4-independent systems (such as ferroptosis suppressor protein 1 (FSP1), dihydroorotate dehydrogenase (DHODH) or GTP cyclohydrolase 1 (GCH1)) depend on GPX4 for survival and therefore are vulnerable to GPX4 inhibition. Conversely, cancer cells with low expression of GPX4 are sensitive to inactivation of components of GPX4-independent systems. The dashed lines indicate that the function of the indicated protein is diminished or blocked in the corresponding context. PLs, phospholipids.

upregulated discoidin domain-containing receptor 2 (DDR2) expression, which may be driven by EMT transcription factors; increased DDR2 promotes ferroptosis sensitivity in cancer cells through the Hippo pathway (the role of which in ferroptosis will be further discussed below)¹¹⁰. Likewise, key enzymes involved in PUFA synthesis, such as elongation of very long-chain fatty acid protein 5 (ELOVL5) and fatty acid desaturase 1 (FADS1), were found to be selectively overexpressed in mesenchymal gastric cancer cells, rendering them particularly susceptible to ferroptosis¹¹¹ (FIG. 2a).

Similar to therapy-resistant mesenchymal-type cancer cells that depend on GPX4, drug-tolerant persistor cancer cells are also highly sensitive to ferroptosis, perhaps because they also exhibit mesenchymal-like

features²¹. Interestingly, CD44, a transmembrane glycoprotein that is highly expressed in mesenchymal cancer cells, mediates hyaluronate-dependent iron endocytosis¹¹², which might promote ferroptosis sensitivity in mesenchymal cancer cells by increasing cellular iron load. Moreover, therapy-resistant dedifferentiated subsets of melanoma cells also show a vulnerability to ferroptosis, possibly because of PUFA accumulation and low levels of GSH¹⁰⁸ (FIG. 2a). In sum, therapy-resistant cancer cells often exhibit altered metabolic states (such as increased PUFA-PLs associated with EMT) that confer a vulnerability to ferroptosis induction.

Certain cancer types are also inherently susceptible to ferroptosis owing to other unique metabolic features (FIG. 2a). As described in a previous section, PUFA-ePLs

synthesized in peroxisomes provide substrates for lipid peroxidation²³. Clear-cell RCC (ccRCC) cells exhibit high levels of PUFA-ePLs, possibly owing to their high expression of alkylglycerone-phosphate synthase (AGPS; a key enzyme involved in PUFA-ePL synthesis), rendering them sensitive to ferroptosis²³. Within small-cell lung cancer (SCLC), non-neuroendocrine SCLC cells are much more sensitive to ferroptosis than are neuroendocrine SCLC cells, at least partly because non-neuroendocrine SCLC cells overexpress ePL synthesis enzymes and have high levels of PUFA-ePLs¹³. Finally, triple-negative breast cancer cells were found to be susceptible to ferroptosis. This susceptibility has been ascribed to several metabolic features of these cells, including their abundant PUFA levels, elevated labile iron pool and attenuated GPX4–GSH defence system^{33,114}. A vulnerability to ferroptosis uncovered in the abovementioned cancer types might provide new therapeutic opportunities for treating these highly intractable diseases.

Genetic mutations in cancer cells. As discussed in BOX 2, the inactivation of tumour suppressors generally promotes ferroptosis resistance. However, in certain cases, tumour suppressor mutations can confer an unexpected vulnerability to ferroptosis. The E-cadherin–neurofibromin 2 (NF2)–Hippo signalling axis presents a remarkable example of this type of vulnerability. Unlike other tumour suppressors (for example, p53, BAP1, KEAP1 and ARF, which promote ferroptosis; see BOX 2), this tumour-suppressive axis suppresses ferroptosis²⁰. E-cadherin-mediated intercellular interactions activate Hippo tumour-suppressive signalling in an NF2-dependent manner, repress the transcriptional activity of Yes-associated protein (YAP) or transcriptional coactivator with PDZ-binding motif (TAZ), and downregulate the expression of several ferroptosis-promoting factors, thereby suppressing ferroptosis (which clearly does not align with the tumour-suppressor function of NF2)^{20,115} (FIG. 2b). Consequently, inactivation of any component in the E-cadherin–NF2–Hippo pathway increases YAP or TAZ expression and/or activity, rendering cancer cells or tumours harbouring mutations in this pathway (such as NF2-mutant mesotheliomas) particularly susceptible to FINS^{20,115}. The Von Hippel-Lindau (VHL) tumour suppressor is another such example. *VHL* is lost in the majority of ccRCCs^{116,117}. Notably, *VHL*-deficient ccRCC cells are susceptible to ferroptosis, and restoration of wild type *VHL* renders them insensitive to ferroptosis¹⁸. Several mechanisms might underlie this unique vulnerability in *VHL*-deficient ccRCCs, prominent among which is the stabilization of hypoxia-inducible factors (HIFs) induced by loss of VHL, which then promote PUFA synthesis through the induction of expression of hypoxia-inducible, lipid droplet-associated protein (HILPDA)^{24,118} (FIG. 2b).

Oncogene activation can also render cancer cells susceptible to ferroptosis. Non-small-cell lung cancer cells with epidermal growth factor receptor (*EGFR*) mutations are highly dependent on cystine and sensitive to ferroptosis induced by *SLC7A11* inhibition or cystine deprivation¹⁹ (FIG. 2b). Interestingly, cystine promotes

cell survival in *EGFR*-mutant cancer cells through GSH-independent mechanisms¹¹⁹, which is in line with recent data showing that cyst(e)ine promotes GPX4 protein synthesis and suppresses ferroptosis partly via GSH-independent mechanisms^{119,120}. As another example, isocitrate dehydrogenase 1 (*IDH1*)-mutated cancer cells exhibit increased levels of the oncometabolite 2-hydroxyglutarate (2-HG), which reduces GPX4 protein levels, sensitizing these cancer cells to ferroptosis¹²¹ (FIG. 2b). Collectively, it appears that oncogene activation and tumour-suppressor inactivation can either suppress or promote ferroptosis, depending on the context. The promotion of ferroptosis can create a vulnerability to ferroptosis for potential therapeutic targeting in corresponding cancer types.

Imbalanced ferroptosis defences. Ferroptosis defence systems can be broadly divided into GPX4-dependent and GPX4-independent arms. Low expression or partial inactivation of one arm can render cancer cells highly dependent on the other for ferroptosis defence, and consequently highly susceptible to ferroptosis induced by the inactivation of the other defence arm (whereas normal cells with both arms intact might be unaffected by inhibition of one defence arm) (FIG. 2c). A therapeutic strategy targeting imbalanced ferroptosis defence would be similar to strategies targeting synthetic lethality, for instance, the use of poly(ADP-ribose) polymerase (PARP) inhibitors to treat *BRCA1*-deficient cancers, which is based on the concept that cells are dependent on two parallel DNA repair pathways, one involving PARP and the other requiring *BRCA1* (REF.¹²²).

In support of this concept, analyses show that cancer cell lines with low expression of *FSP1*, *DHODH* or *GCH1* are in general more vulnerable to GPX4 inhibitors than those with high expression of the corresponding gene (indeed, *FSP1* was identified as the gene with the most striking such correlation in the [Cancer Therapeutics Response Portal](#))^{5–9}. Furthermore, GPX4 genetic ablation reduced tumour growth more potently in *FSP1* knockout xenograft tumours than in *FSP1* wild type counterparts, and this reduction in tumour growth was attributed to ferroptosis induction⁵. Due to the unsuitability of current GPX4 inhibitors for in vivo studies (see further discussion in Perspective), whether this observation also applies to GPX4 inhibitor treatment of tumours remains to be established. Conversely, cancer cells with low expression of GPX4 are more dependent on *FSP1* or *DHODH* for ferroptosis defence, and are therefore more sensitive to ferroptosis induced by inactivation of *FSP1* or *DHODH* than are those with high GPX4 expression^{5,6,9}. *DHODH* inhibitors were found to suppress the growth of GPX4^{low} xenograft tumours more potently than that of GPX4^{high} xenograft tumours⁹. Likewise, low expression of GPX4 (in concert with high expression of *ALOX5*) renders germinal centre B cell-like diffuse large B cell lymphoma cells vulnerable to ferroptosis induced by dimethyl fumarate (a compound approved for psoriasis treatment that depletes GSH through direct GSH succinylation)¹²³. These recent findings suggest that the expression levels of genes involved in ferroptosis defence can be explored as biomarkers

Cyst(e)inase
Engineered enzymes that
degrade extracellular cysteine
and cystine.

to select patients for cancer treatment with FINs that target other ferroptosis defence arms.

Ferroptosis in the tumour microenvironment

Recent studies have also revealed that the tumour microenvironment (TME), particularly its immune cells, dictates whether tumour-cell ferroptosis will occur. CD8⁺ cytotoxic T cells, the major executors of antitumour immunity in the TME, secrete interferon-γ (IFNγ), which subsequently inhibits cystine uptake in cancer cells through the downregulation of SLC7A11 expression, thereby augmenting lipid peroxidation and ferroptosis in tumours²⁸ (FIG. 3). Interestingly, IFNγ has also been shown to suppress SLC7A11-mediated cystine transport in macrophages¹²⁴, suggesting that IFNγ can regulate SLC7A11 expression and/or activity in both cancer and non-cancer contexts. Furthermore, immune checkpoint inhibitors (ICIs) and cyst(e)inase together potentiated T cell-mediated antitumour immune responses by synergistically promoting tumour ferroptosis, suggesting that ferroptosis is important in T cell-mediated antitumour activity and that blocking SLC7A11-mediated cystine uptake in combination with ICIs is a potential therapeutic strategy for cancer²⁸. Of note, ferroptotic cancer cells can release several immunostimulatory signals, such as high mobility group box 1 (HMGB1), calreticulin, ATP and phosphatidylethanolamine, which promote dendritic cell maturation, increase the efficiency of macrophages in the phagocytosis of ferroptotic cancer cells and further enhance the infiltration of CD8⁺ T cells into tumours^{125–128} (FIG. 3). Specifically,

by releasing immunostimulatory signals, early ferroptotic cells (after 1 or 3 h of treatment with a GPX4 inhibitor) can promote the phenotypic maturation of dendritic cells and elicit a vaccination-like effect to activate antitumour immunity¹²⁷. Such evidence supports the concept that ferroptosis may act as a form of immunogenic cell death.

Ferroptosis induction in some immunosuppressive cells can also augment antitumour immunity (FIG. 3). Regulatory T (T_{reg}) cells, an immunosuppressive subset of CD4⁺ T cells that hinder protective immune surveillance against tumours, are relatively resistant to ferroptosis, possibly owing to GPX4 induction in activated T_{reg} cells^{129,130}. Correspondingly, T_{reg} cell-specific deletion of *Gpx4* triggers ferroptosis in T_{reg} cells, contributing to antitumour immunity¹³⁰. Similarly, myeloid-derived suppressor cells (MDSCs) with immunosuppressive functions exhibit resistance to ferroptosis that is driven by N-acylsphingosine amidohydrolase 2 (ASA2)-mediated inhibition of the p53-haeme oxygenase 1 (HMOX1) axis¹³¹; consequently, targeting ASA2 to induce ferroptosis in MDSCs increases the activation of tumour-infiltrating cytotoxic CD8⁺ T cells and promotes tumour suppression¹³¹. In addition, tumour-associated macrophages (TAMs) predominantly display the M2-like phenotype to suppress antitumour immunity¹³². Notably, immunosuppressive M2-like TAMs are more vulnerable to ferroptosis induced by inhibition of GPX4 owing to their lack of inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO[•]) generation (NO[•] can detoxify lipid peroxides) than are the M1-like TAMs that promote antitumour immunity^{126,133}. Therefore, inducing

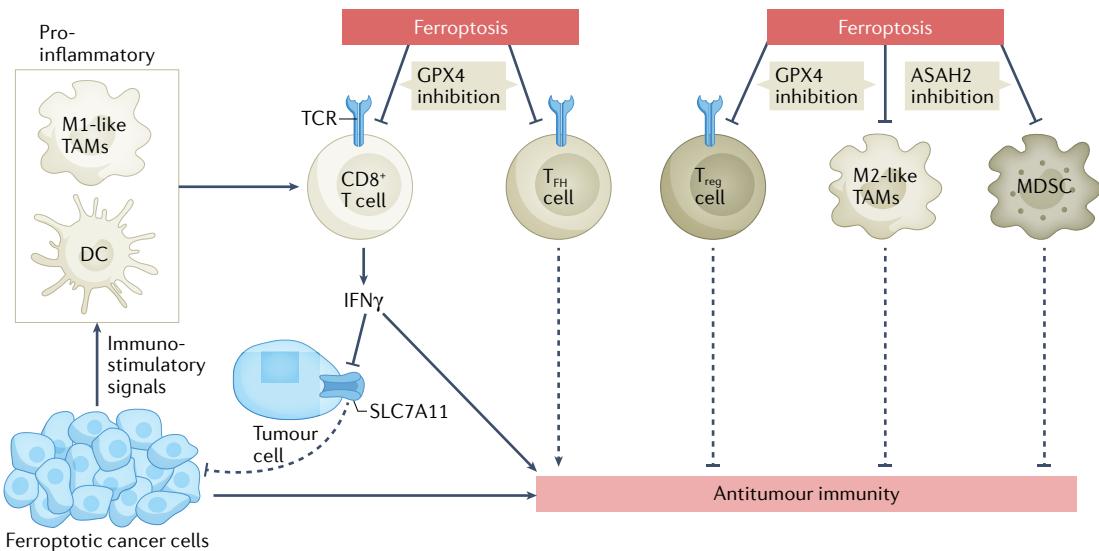


Fig. 3 | Role of ferroptosis in antitumour immunity. Ferroptosis has a dual role to play in antitumour immunity, dependent on the nature of the immune cell. Boosting antitumour immunity, interferon-γ (IFNγ) secreted by CD8⁺ T cells promotes cancer cell ferroptosis by repressing solute carrier family 7 member 11 (SLC7A11) expression in cancer cells. In turn, ferroptotic cancer cells release immunostimulatory signals that promote dendritic cell (DC) maturation and increase the efficiency of macrophages, particularly M1-like tumour-associated macrophages (TAMs), to phagocytose ferroptotic cancer cells. This further strengthens CD8⁺ T cell-mediated tumour suppression. In addition, several types of immunosuppressive cells, including regulatory T (T_{reg}) cells, myeloid-derived suppressor cells (MDSCs) and M2-like TAMs, are impaired by ferroptosis induction mediated by inhibition of glutathione peroxidase 4 (GPX4) or N-acylsphingosine amidohydrolase 2 (ASA2), thereby augmenting antitumour immunity. However, CD8⁺ T cells and some T helper cell subsets, such as T follicular helper (T_{FH}) cells, are also susceptible to ferroptosis, which compromises the contribution of ferroptosis to antitumour immunity. The dashed lines indicate that the function of the indicated cell or protein is diminished or blocked in the corresponding context.

Transsulfuration pathway
A metabolic pathway that transfers sulfur from homocysteine to cysteine, leading to cysteine biosynthesis.

ferroptosis in M2-like TAMs without affecting M1-like TAMs is a potential strategy to overcome the immunosuppressive TME and augment the effects of cancer immunotherapy¹³³.

However, emerging evidence also indicates that ferroptosis has a tumour-promoting effect in the context of tumour immunity. *Gpx4*-deficient T cells derived from T cell-specific *Gpx4* knockout mice rapidly accumulate lipid peroxides in cell membranes upon T cell activation and subsequently undergo ferroptosis¹³⁴. Furthermore, substantial lipid peroxides are detected in CD8⁺ T cells derived from tumours but not in those from lymph nodes, suggesting that ferroptosis may be a metabolic vulnerability of tumour-specific CD8⁺ T cells¹³⁵. Ferroptosis in this context would presumably dampen antitumour immunity and promote tumour growth. Supporting this idea, CD36 mediates fatty acid uptake by tumour-infiltrating CD8⁺ T cells in the TME, and increased CD36 expression induces lipid peroxidation and ferroptosis in CD8⁺ T cells, thereby compromising antitumour immunity¹³⁶. Genetic ablation of CD36 or blocking ferroptosis in CD8⁺ T cells effectively restores their antitumour activity, whereas CD8⁺ T cells treated with GPX4 inhibitors undergo ferroptosis and thus exert impaired antitumour effects *in vivo*¹³⁶. Furthermore, T follicular helper (T_{FH}) cells, a subset of CD4⁺ T cells that favours antitumour immunity, are highly susceptible to ferroptosis, and GPX4 expression is necessary for their survival and function¹³⁷ (FIG. 3). However, it should be noted that *Slc7a11* knockout in mice or cystine deprivation *in vivo* does not decrease the viability or antitumour effects of T cells¹³⁸, which may explain the synergistic augmentation of antitumour immunity by targeting SLC7A11 in combination with ICIs²⁸. The mechanisms underlying the differential effects of *GPX4* deletion versus *SLC7A11* deletion on T cell function remain elusive but might relate to the low expression (and likely a non-essential role) of SLC7A11 in T cells^{139,140}.

Targeting ferroptosis in cancer therapy

In recent years, FINs (BOX 1) have garnered considerable interest in cancer research owing to their enormous therapeutic potential. Moreover, a variety of nanomaterials have been developed to induce ferroptosis locally or to enhance the activity of FINs³⁰. Furthermore, growing evidence suggests that ferroptosis at least partly mediates the tumour-suppressive effects of several conventional cancer therapies, including RT^{25,141–143}, chemotherapy²⁶, targeted therapy^{27,144} and immunotherapy²⁸, and that FINs could potentiate the efficacy of these therapies by boosting tumour ferroptosis. As discussed below, inducing ferroptosis with FINs could be a promising therapeutic strategy to eliminate cancers with specific characteristics (TABLE 1).

Exploiting ferroptosis vulnerabilities. As outlined in the preceding section, ferroptosis represents a vulnerability in certain cancer types, and targeting these vulnerabilities by inducing ferroptosis provides opportunities for cancer treatment. Notably, in several cancer types (such as lung and breast cancers), cancer cells appear to be more sensitive to ferroptosis than their corresponding

normal epithelial cells *in vitro*¹⁴². These data highlight the existence of appropriate therapeutic windows that would allow selective ferroptosis induction in tumours while sparing normal tissues.

Re-sensitizing resistant tumours to ferroptosis. As previously discussed, diverse ferroptosis escape mechanisms confer ferroptosis resistance in cancer cells. Disrupting the mechanisms that drive ferroptosis evasion can re-sensitize ferroptosis-resistant cancer cells or tumours to ferroptosis. Ferroptosis resistance mediated by certain genes with oncogenic activities (BOX 3) can be overcome by inhibiting expression or activity of the protein products of the genes themselves. For example, DJ1 depletion by disrupting the activity of S-adenosyl homocysteine hydrolase (SAHH) in the transsulfuration pathway re-sensitizes xenograft tumours to FINs that block SLC7A11 (REF.¹⁴⁵). The transsulfuration pathway is constitutively active in conducting de novo cysteine synthesis in some cancer cells, whereas the expression of transsulfuration enzymes can be induced by cystine starvation in others^{145–147}. Therefore, while cancer cells mainly rely on SLC7A11-mediated cystine uptake to generate intracellular cysteine, the transsulfuration pathway still significantly contributes to the intracellular cysteine pool in some cancer cells and protects them from cystine starvation-induced ferroptosis^{146,147}. Consequently, targeting enzymes involved in the transsulfuration pathway, such as the DJ1–SAHH axis, cystathione β -synthase (CBS) and glycine N-methyltransferase (GNMT), can potentiate the susceptibility of tumours to FINs that block SLC7A11 (REFS^{145,146,148}). As another example, pyruvate dehydrogenase kinase 4 (PDK4) inhibition by accelerating pyruvate oxidation and stimulating fatty acid synthesis can re-sensitize xenograft tumours to FINs that block SLC7A11 (REF.¹⁴⁹).

Oncogene-induced ferroptosis resistance can also be mediated through downstream effectors that can be targeted to reverse ferroptosis resistance. For instance, PI3K promotes ferroptosis resistance mainly through mTOR complex 1 (mTORC1), and the combination of mTORC1 inhibitors with FINs that block SLC7A11 exerts potent tumour-suppressive effects in *PIK3CA*-mutant or *PTEN*-mutant xenograft tumours¹⁷. Cysteine depletion by SLC7A11 inhibitors or cyst(e)inase exhibits striking efficacy against *KRAS*-mutant tumours, including those from genetically engineered mouse models, by impairing the enhanced ferroptosis defence (that is, increased levels of intracellular cysteine and GSH) on which *KRAS*-mutant tumours depend^{102,103,150}. Overall, targeting key oncogenic pathways that confer ferroptosis resistance represents an important strategy for cancer therapy.

Combining FINs with conventional cancer therapies.

It is increasingly appreciated that diverse forms of conventional cancer therapy can trigger ferroptosis. Therefore, boosting ferroptosis induced by these therapies (for instance, with FINs) could further strengthen their therapeutic efficacy. RT induces ferroptosis through multiple parallel mechanisms¹⁵¹. In response to RT, cancer cells evolve adaptive responses, such as upregulating

Table 1 | Strategies for targeting ferroptosis in cancer therapy

| Strategy | Classification | Feature | Tumour type | Target or agent |
|--|--|--|--|--|
| Exploiting the vulnerability of tumours to ferroptosis | Cancer cells with specific metabolic features | EMT | Multiple cancers | GPX4 inhibitors, HMGCR inhibitors ^{22,111} |
| | | Drug-tolerant persister | Multiple cancers | GPX4 inhibitors ²¹ |
| | | Dedifferentiation | Melanoma | SLC7A11 inhibitors, GPX4 inhibitors ¹⁰⁸ |
| | | ccRCC | RCC | GPX4 inhibitors ²³ |
| | | Non-NE SCLC | SCLC | SLC7A11 inhibitors, GPX4 inhibitors ¹¹³ |
| | Cancer cells with certain genetic mutations | TNBC | Breast cancer | SLC7A11 inhibitors, GPX4 inhibitors ¹¹⁴ |
| | | Mutations in E-cadherin–NF2–Hippo axis | Multiple cancer types | SLC7A11 inhibitors, GPX4 inhibitors ^{20,115} |
| | | VHL deficiency | RCC | GPX4 inhibitors, inhibition of glutathione synthesis (erastin, BSO) ^{24,118} |
| | | EGFR mutation | NSCLC | Cysteine depletion ¹¹⁹ |
| | | IDH1 mutation | Multiple cancer types | SLC7A11 inhibitors ¹²¹ |
| Re-sensitizing resistant tumours to ferroptosis | Cancer cells with imbalanced ferroptosis defence | GPX4 low | Multiple cancer types | FSP1 inhibitors, DHODH inhibitors, GCH1 inhibitors, BH ₄ depletion ^{5–9} |
| | | FSP1, DHODH or GCH1 low | Multiple cancer types | SLC7A11 inhibitors, GPX4 inhibitors ^{5–9} |
| | | iPLA ₂ β overexpression | p53 wild type cancers | iPLA ₂ β inhibition + TBH ⁹⁰ |
| | | Adipokine chemerin upregulation | RCC | Chemerin monoclonal antibody ⁹² |
| | | NFS1 overexpression | Lung cancer | NFS1 inhibition + BSO, erastin or TBH ⁹⁵ |
| | De-restriction of the labile iron availability | Frataxin overexpression | Multiple cancer types | Frataxin inhibition + erastin ⁹⁶ |
| | | CISD2 overexpression | Head and neck cancer | Pioglitazone (CISD2 inhibitor) + sulfasalazine (SLC7A11 inhibitor) ⁹⁷ |
| | | Prominin 2 upregulation | Breast cancer | Prominin 2 inhibition + SLC7A11 inhibitors or GPX4 inhibitors ⁹⁸ |
| | | SLC7A11 upregulation | KRAS mutation | SLC7A11 inhibitors ^{102,103} |
| | | GPX4 upregulation | Cancers chronically exposed to 27-HC | GPX4 inhibition ⁹³ |
| Preventing the upregulation of ferroptosis defence systems | FSP1 or GCH1 high expression | Multiple cancer types | FSP1 inhibitors, GCH1 inhibitors, BH ₄ depletion ^{5–8} | |
| | | SQLE deficiency | ALK ⁺ anaplastic large cell lymphoma | GPX4 inhibitors + inhibition of squalene synthesis (atorvastatin, zaragozic acid, SQS inhibition) ¹⁰⁵ |
| | | PIK3CA-activating mutations or PTEN deletion | Multiple cancer types | mTORC1 inhibitors + GPX4 inhibitors or SLC7A11 inhibitors ¹⁷ |
| | | NEDD4 overexpression | Melanoma | NEDD4 inhibition + SLC7A11 inhibitors ¹⁶⁹ |
| | | DJ1 overexpression | Multiple cancer types | DJ1 inhibition + SLC7A11 inhibitors or GPX4 inhibitors ¹⁴⁵ |
| | Abrogation of oncogenic activation-mediated ferroptosis resistance | PDK4 overexpression | Pancreatic ductal adenocarcinoma | PDK4 inhibitors + SLC7A11 inhibitors ¹⁴⁹ |
| | | Chemotherapy, immunotherapy or immunotherapy in combination with RT | Multiple cancer types | SLC7A11 inhibitors + cisplatin, doxorubicin, ICIs, or ICIs + RT ^{26,28,143,153,154} |
| | | SLC7A11 or GPX4 induction upon RT | NSCLC | RT + SLC7A11 inhibitors or GPX4 inhibitors ²⁵ |
| | | GPX4 induction upon treatment with gemcitabine | Pancreatic ductal adenocarcinoma | Gemcitabine + SLC7A11 inhibitors ¹⁵² |
| | | Acquired radioresistance, intrinsic radioresistance (TP53 or KEAP1 mutation) | NSCLC | RT + SLC7A11 inhibitors or GPX4 inhibitors ^{25,142,155} |
| Combining FNs with conventional cancer therapies | Sensitizing conventional cancer therapies | Acquired chemoresistance (cisplatin or docetaxel) | Head and neck cancer, ovarian cancer | SLC7A11 inhibitors + cisplatin or docetaxel ^{157,158} |
| | | TYRO3-mediated anti-PD1 or anti-PDL1 therapy resistance | Breast cancer | TYRO3 inhibitors + anti-PD1 ¹⁵⁹ |
| | | MT1G-mediated sorafenib resistance | Hepatocellular carcinoma | MT1G inhibitors + sorafenib ¹⁶⁰ |
| | Overcoming resistance to conventional cancer therapies | | | |
| | | | | |

27-HC, 27-hydroxycholesterol; ALK, anaplastic lymphoma kinase; BSO, buthionine sulfoximine; BH₄, tetrahydrobiopterin; ccRCC, clear cell renal cell carcinoma; CISD2, CDSH iron–sulfur domain-containing protein 2; DHODH, dihydroorotate dehydrogenase; EMT, epithelial-to-mesenchymal transition; FNs, ferroptosis inducers; FSP1, ferroptosis suppressor protein 1; GCH1, GTP cyclohydrolase 1; GPX4, glutathione peroxidase 4; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; ICIs, immune checkpoint inhibitors; iPLA₂β, calcium-independent phospholipase A₂β; MT1G, metallothionein 1G; mTORC1, mTOR complex 1; NE, neuroendocrine; NCSLC, non-small-cell lung cancer; NF2, neurofibromin 2; PD1, programmed cell death protein 1; PDK4, pyruvate dehydrogenase kinase 4; PUFA-PL, polyunsaturated fatty acid-containing phospholipid; RCC, renal cell carcinoma; RT, radiotherapy; SCLC, small-cell lung cancer; SLC7A11, solute carrier family 7 member 11; SQLE, squalene monooxygenase; SQS, squalene synthase; TBH, tert-butyl-hydroperoxide; TNBC, triple-negative breast cancer.

SLC7A11 or GPX4 expression, to antagonize RT-induced ferroptosis²⁵. Consequently, FINs targeting either SLC7A11 or GPX4 in combination with RT can radiosensitize cancer cells or xenograft tumours by potentiating ferroptosis^{25,141}. Similarly, gemcitabine, a chemotherapeutic agent, induces GPX4 expression and activity; GPX4 inhibition counteracts this effect, increasing the sensitivity of cancer cells and xenograft tumours to gemcitabine by inducing ferroptosis¹⁵². Likewise, FINs targeting SLC7A11 can sensitize cancer cells to chemotherapy (for example, cisplatin and doxorubicin)^{153,154}, immunotherapy (for example, ICIs)²⁸ and immunotherapy in combination with RT¹⁴³.

Notably, for some cancer types with intrinsic or acquired therapy resistance, inducing ferroptosis with FINs could restore their sensitivity to conventional therapies. Mutations in the tumour suppressors TP53 or KEAP1 dampen RT-induced ferroptosis by upregulating SLC7A11 and other mechanisms, resulting in intrinsic radioresistance^{25,142}. Cancer cells with acquired radioresistance also exhibit SLC7A11 upregulation and resistance to ferroptosis^{142,155,156}. Consequently, reactivation of RT-induced ferroptosis by FINs can restore radiosensitivity in these cancer cells and xenograft tumours^{142,155}. Likewise, chemoresistance in certain cancer types can be overcome by FINs. It was reported that cisplatin resistance in head and neck squamous cell carcinoma cells and corresponding xenograft tumours could be abrogated by adding FINs targeting SLC7A11 (REF.¹⁵⁷). In addition, FINs that inhibit SLC7A11 reversed docetaxel resistance in ovarian cancer cells *in vitro*¹⁵⁸.

In the examples discussed above, FINs were combined with conventional therapies to overcome therapy resistance. Likewise, specific inhibitors can be combined with conventional therapies to boost ferroptosis induction in tumours and reverse therapy resistance. Tumours with high TYRO3 expression are resistant to anti-PD1 or anti-PDL1 therapy, while metallothionein 1G (MT1G) induction renders cancer cells resistant to the multi-kinase inhibitor sorafenib. It was recently revealed that therapeutic resistance induced by these proteins is partly due to ferroptosis blockade and can be overcome by restoring ferroptosis through a combination of anti-PD1 or anti-PDL1 therapy with TYRO3 inhibitors or sorafenib with MT1G inhibitors *in vivo*^{159,160}.

Taken together, resistance to RT, chemotherapy, immunotherapy and other targeted therapies has been linked to ferroptosis resistance and this phenotype can be reversed by restoring ferroptosis with FINs or other inhibitors. It is important to note that the combination of FINs, especially class I FINs that inhibit SLC7A11, with conventional therapy appears to cause relatively limited toxic effects in normal cells¹⁴² and shows good tolerability in preclinical models^{25,28,141–143,159,160}, further supporting the clinical application of ferroptosis-inducing combination therapeutic strategies. Several clinical trials are currently under way to test the efficacy and safety of FINs or anticancer drugs with ferroptosis-inducing activity, either alone or in combination with conventional therapy, in patients with cancer (for example, NCT04205357 (REF.¹⁶¹), NCT04092647 (REF.¹⁶²), NCT02559778 (REF.¹⁶³), NCT03247088 (REF.¹⁶⁴)).

Perspective

Ferroptosis is a form of metabolically regulated cell death. Metabolism in cancer cells is substantially rewired to meet their increased bioenergetic and biosynthetic needs and to support their rapid proliferation. This metabolic reprogramming often induces unique metabolic features, such as an enrichment of PUFA-PLs, an overload of iron and imbalanced ferroptosis defence systems, that can create a targetable vulnerability to ferroptosis and represents an exciting opportunity for the discovery of new therapeutic targets in cancer. In addition, for tumours that exhibit intrinsic or acquired ferroptosis resistance, targeting the underlying resistance mechanisms can restore their vulnerability to ferroptosis. Therapeutic efficacy can be further enhanced by combining FINs with conventional therapies that can induce ferroptosis; importantly, such combination therapies have shown good synergism and tolerability in preclinical models. However, thorough histological and pharmacological analyses remain imperative to evaluate potential toxic effects of FINs in normal tissues and to determine the optimal drug dosing and scheduling in patients. To realize the full potential of ferroptosis-inducing strategies in cancer therapy, several additional challenges remain to be tackled in future investigations.

GPX4 constitutes the most powerful defence against ferroptosis, and several types of therapy-resistant tumours are remarkably vulnerable to GPX4 inhibition^{22–24}, highlighting the importance of GPX4 inhibitors in targeting ferroptosis vulnerability in cancer. The GPX4 covalent inhibitor ML210 and its recently designed derivatives (such as JKE-1674) are more selective than are RSL3 and ML162 (BOX 1); however, most of these inhibitors have exhibited poor (or unclear) pharmacological properties in animal models, limiting their potential for clinical translation¹⁶⁵. Developing and optimizing GPX4-targeting agents with improved pharmacokinetics and selectivity remains a major barrier to employing GPX4 inhibition in cancer therapy. In this regard, several currently available and *in vivo*-stable anticancer agents with GPX4-inhibitory activity, such as withaferin A and altretamine, may offer an alternative to address this problem^{166,167}. In addition, considering that *Gpx4* is an essential gene in the mouse^{49,72}, it also remains to be determined whether pharmacological inhibition of GPX4 can selectively kill tumours without inducing extensive toxicity in normal tissues and intolerable side effects in patients.

In addition to cancer cells, several cell types in the TME, including immune cells that promote or suppress antitumour immunity, might also be susceptible to ferroptosis. Therefore, how to balance the ferroptosis vulnerabilities of cancer cells, antitumour immune cells and immunosuppressive cells remains a critical obstacle. To address this issue, it will be important to comprehensively understand the mechanisms underlying the differential sensitivities of cancer cells and various immune cells to ferroptosis.

Furthermore, there remains an urgent need to develop predictive biomarkers that can accurately predict tumour responses to ferroptosis induction, especially those that can be tested directly in patients' body fluids and biopsy specimens. Such tools will be essential

for the stratification of patients with cancer for treatment with ferroptosis-inducing therapies.

More in-depth understanding of ferroptosis mechanisms will continue to provide key insights into targeting ferroptosis in cancer. As noted above, whether cytosolic GPX4 can substantially localize within mitochondria and suppress mitochondrial lipid peroxidation remains to be clarified. The recently proposed compartmentalization model⁹ also suggests that additional ferroptosis defence systems might exist in other organelles. Another key question in ferroptosis research is whether lipid peroxidation on the plasma membrane represents the final step or whether there are additional as-yet-unknown downstream steps in triggering plasma membrane rupture and ferroptotic cell death. The observation that cytosolic GPX4 (which can detoxify lipid peroxides accumulated on the plasma membrane) fails to suppress ferroptosis induced by mitochondrial lipid peroxidation in GPX4-knockout cells treated with DHODH inhibitors⁹ indicates additional ferroptosis execution mechanisms downstream of plasma membrane lipid peroxidation. Understanding these fundamental questions might help to identify new targets in cancer therapy.

Finally, it is believed that proteins involved in PUFA-PL synthesis and peroxidation (such as ACSL4 or POR)

play a passive role in ferroptosis execution by providing a constitutive supply of PUFA-PLs or ROS for lipid peroxidation (in contrast to cell death executioner proteins involved in other cell death mechanisms, such as caspase, MLKL and gasdermin D, which play an active role in inducing cell death in response to physiological cues and, correspondingly, their activation is tightly controlled by upstream signalling). This view was challenged by a very recent study showing that initial ferroptotic stress activates protein kinase C β isoform II (PKC β II), which then phosphorylates ACSL4 and promotes ACSL4-mediated PUFA-PL synthesis and subsequent ferroptosis, thereby forming a positive feedforward loop to amplify ferroptosis¹⁶⁸. Attenuation of the PKC β II-ACSL4 signalling axis dampens the efficacy of immunotherapy by suppressing tumour ferroptosis¹⁶⁸. This study will inspire future investigations aimed at understanding additional regulatory mechanisms involved in ferroptosis execution and their relevance to cancer therapy. We envision that the next few years will witness exciting new findings that will translate our mechanistic understanding of this intriguing cell death mechanism into effective cancer therapies.

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Author contributions

G.L. and B.G. researched data for the article and contributed substantially to discussion of the content. All authors wrote

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Competing interests

B.G. is an inventor on patent applications involving targeting ferroptosis in cancer therapy. G.L. and L.Z. declare no competing interests.

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