HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2023 June 06.

Published in final edited form as:

Nat Rev Cancer. 2022 July ; 22(7): 381–396. doi:10.1038/s41568-022-00459-0.

Targeting ferroptosis as a vulnerability in cancer

Guang Lei[1], **Li Zhuang**[1], **Boyi Gan**[1,2,*]

[1]Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

[2]The University of Texas MD Anderson UTHealth Graduate School of Biomedical Sciences, Houston, TX, USA.

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.

summary

In recent years, research in the field of ferroptosis in cancer has risen steeply and this is in part owing to its potential to be targeted. In this Review, Lei et al. provide an up-to-date synthesis of the roles and mechanisms of ferroptosis in tumour growth and progression including its function in tumour immunity, highlighting it as a vulnerability that can be exploited for cancer therapy.

Introduction

Ferroptosis, a term coined by Stockwell and colleagues in 2012[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

*Correspondence: bgan@mdanderson.org.

Author Contributions

G.L. and B.G. researched data for the article and contributed substantially to discussion of the content. All authors wrote the article and reviewed and/or edited the manuscript before submission.

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.

of ferroptosis[3] 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 which differ from 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 one hand, multiple cancer-associated signalling pathways have been shown to govern ferroptosis in cancer cells[14]. 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 cells[9]. 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.

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[13,32,33] (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 [PE] or adrenic acid-PE)[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[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].

PUFA-PLs are particularly susceptible to peroxidation due to the presence of bis-allylic moieties in PUFAs[41]. The peroxidation of PUFA-PLs is primarily catalysed by nonenzymatic autoxidation driven by the **Fenton reaction** with iron as a catalyst[41–43] (Fig. 1b). Cytochrome P450 oxidoreductase (POR)- 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 hydrogen peroxide (H_2O_2)[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 mice[49]. Monounsaturated fatty acids (MUFAs), such as oleic acid and palmitoleic acid, are not readily peroxidised owing to their lack of bis-allylic moieties[50]. In contrast to PUFAs, MUFAs 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 nonenzymatic Fenton reaction for direct peroxidation of PUFA-PLs[41,43], but also by acting as an 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 export[53]. 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 ferroptosis[53].

Mitochondrial metabolism.

Several metabolic processes in mitochondria have important roles in triggering ferroptosis[31] (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 H_2O_2 by superoxide dismutase[57]. H_2O_2 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 (i.e., 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 the TCA cycle (such as glutaminolysis) in mitochondria[59], which drive ferroptosis likely through promoting 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 ferroptosis[31].

Ferroptosis defence mechanisms

Ferroptosis defence mechanisms involve cellular antioxidant systems that directly neutralize lipid peroxides. Studies of ferroptosis defence mechanisms have 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 4 such ferroptosis defence systems with distinctive subcellular localizations (Fig. 1).

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 3 isoforms with distinctive subcellular localizations, namely cytosolic, mitochondrial, and nuclear GPX4s. 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 cytosolic GPX4, but not the mitochondrial or nuclear isoform, is required for embryonic development[72–75] and because reexpression of cytosolic GPX4, but not mitochondrial or nuclear GPX4, significantly suppresses cell death induced by *Gpx4* deletion in mouse embryonic fibroblasts[76]. 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 that is derived from glycine, glutamate, and cysteine, with cysteine being the rate-limiting precursor[77,78]. Most cancer cells obtain intracellular cysteine primarily through **system x_c^-**-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 x_c^- [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 ferroptosis[22], suggesting the existence of additional ferroptosis defence mechanisms.

The FSP1–$CoQH_2$ 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 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** ($CoQH_2$)[5,6] (Fig. 1b). Apart from its well-known function in mitochondrial electron transport, $CoQH_2$ can also trap lipid peroxyl radicals, thereby suppressing lipid peroxidation and ferroptosis. Therefore, it has been proposed that FSP1 exerts its potent antiferroptosis activity through generating the nonmitochondrial $CoQH_2$ pool as radical-trapping antioxidants[5,6]. It should be noted that CoQ is mainly synthesized in mitochondria[83] but has been detected in nonmitochondrial membranes, including the plasma membrane[85–88]. The sources of the nonmitochondrial CoQ utilized by FSP1 for ferroptosis defence remain to be established.

The DHODH–$CoQH_2$ 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 peroxidation[9]. DHODH is an enzyme involved in pyrimidine synthesis that can reduce CoQ to $CoQH_2$ in the inner mitochondrial membrane (Fig. 1b). When GPX4 is acutely inactivated, the flux through DHODH is significantly increased, resulting in enhanced $CoQH_2$ generation that neutralises lipid peroxidation and defends against

ferroptosis in mitochondria[9]. 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 so[9], 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 2 major divisions, the GPX4 system and the $CoQH_2$ system, with each of these further subdivided into arms located in nonmitochondrial and mitochondrial compartments (i.e., cytosolic and mitochondrial GPX4 in the GPX4 system, versus FSP1 and DHODH in the $CoQH_2$ 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- or other compartment-localized defence systems from entering into 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 mitochondria[74]. 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 $CoQH_2$, 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 or even promotes resistance to cystine starvation-induced ferroptosis[62]. Conceivably, the presumed antiferroptosis function of complex I or II (by producing mitochondrial $CoQH_2$) might be offset by its proferroptosis functions (through producing ROS or ATP; see subsection on mitochondrial metabolism). The potential role of additional $CoQH_2$-producing enzymes localized in mitochondria, such as electron transfer flavoprotein dehydrogenase (ETFDH), in ferroptosis regulation awaits further research.

The GCH1–BH_4 system.

Recent studies identified GTP cyclohydroxylase-1 (GCH1) as another critical regulator of ferroptosis[7,8]. Tetrahydrobiopterin (BH_4) is a cofactor of aromatic amino acid hydroxylases and other enzymes, and GCH1 mediates the rate-limiting reaction in the BH_4 biosynthesis pathway[89]. BH_4 is another radical-trapping antioxidant capable of trapping lipid peroxyl radicals, and its function in inhibiting ferroptosis appears to be independent of its role as a cofactor[8]. It was proposed that GCH1 suppresses ferroptosis through generating BH_4 as a radical-trapping antioxidant as well as via GCH1-mediated production of $CoQH_2$ 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 (SFA) tail at

the *sn*-1 position) (Fig. 1b). The subcellular compartment wherein the GCH1–BH_4 system operates remains to be defined.

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 and 3). Tumours have evolved at least 3 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 peroxidised PUFA-PL levels in cancer cells has been linked to ferroptosis evasion and enhanced tumour growth. Calcium-independent phospholipase $A_2\beta$ ($iPLA_2\beta$), a member of the $iPLA_2$ family, is overexpressed in some human cancers. $iPLA_2\beta$ was recently shown to facilitate cancer cell escape from ferroptosis by hydrolysing peroxidised PLs, and $iPLA_2\beta$ 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 downregulating the levels of peroxidised PUFA-PLs and promoting ferroptosis evasion[92].

Recent findings highlight that ferroptosis evasion through modulating 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 and low levels of free iron in the lymphatic fluid; oleic acid protects melanoma cells from ferroptosis in an ACSL3-dependent manner[18] (as discussed in 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 blood[18]. Likewise, under conditions of **hypercholesterolemia**, cancer cells exhibit superior metastatic capabilities *in vivo*, which may be attributable to ferroptosis resistance caused by increased accumulation of **lipid droplets** and MUFAs in these cells[93].

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 pool[94]. 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 ferroptosis[95]. Frataxin, another protein involved in ISC assembly, is upregulated in different cancer types and was shown to

promote cancer cell resistance to ferroptosis and, thus, xenograft tumour growth[96]. 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 ISCs and decreasing free iron levels[97]. In addition, when breast cancer cells detach from the extracellular matrix, they can upregulate prominin 2 expression to promote the formation of ferritin-containing multivesicular bodies that export iron out of cells, thereby facilitating ferroptosis evasion *in vitro*[98]. 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 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 vivo*[93].

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 vitro*[7]. FSP1- and DHODH-mediated ferroptosis defence centres on CoQ, whose synthesis is derived from the **mevalonate pathway**[83]. 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 vivo*[105]. This mechanism provides a unique advantage for ALK$^+$ anaplastic large-cell lymphoma growth under conditions of oxidative stress[105].

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 3 such ferroptosis-based vulnerabilities in cancer cells, namely those induced by metabolic reprogramming, genetic mutations, and an imbalance in ferroptosis defences (Fig. 2a–c).

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 high expression of zinc finger E-box binding homeobox 1 (ZEB1), a driver of **epithelial-to-mesenchymal transition** (EMT) and lipogenic factor[109]. 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 ferroptosis[22]. Consistently, certain recurrent breast cancer cells with mesenchymal features exhibit 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 these cancer cells particularly susceptible to ferroptosis[111] (Fig. 2a).

Similar to therapy-resistant mesenchymal-type cancer cells that depend on GPX4, drug-tolerant persister cancer cells also are highly sensitive to ferroptosis, perhaps because they also exhibit mesenchymal-like features[21]. Interestingly, CD44, a transmembrane glycoprotein that is highly expressed in mesenchymal cancer cells, mediates hyaluronate-dependent iron endocytosis[112], which might promote ferroptosis sensitivity in mesenchymal cancer cells through 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 GSH[108] (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 peroxidation[23]. 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 these cells sensitive to ferroptosis[23]. 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-ePLs[113]. Finally, triple-negative breast cancer (TNBC) 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, 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 (e.g., p53, BAP1, KEAP1 and ARF (p14), which promote ferroptosis; see Box 2), this tumour-suppressive axis suppresses ferroptosis[20]. 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 ferroptosis[118]. Several mechanisms might underlie this unique vulnerability in *VHL*-deficient ccRCCs, prominent among which is stabilization of hypoxia inducible factors (HIFs) induced by loss of VHL, which then promote PUFA synthesis through inducing the 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 (NSCLC) cells with epidermal growth factor receptor (*EGFR*) mutations are highly dependent on cystine and sensitive to ferroptosis induced by SLC7A11 inhibition or cystine deprivation[119] (Fig. 2b). Interestingly, cystine promotes cell survival in *EGFR*-mutant cancer cells through GSH-independent mechanisms[119], 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 ferroptosis[121] (Fig. 2b). Collectively, it appears that oncogene activation and tumour-suppressor inactivation can either suppress or promote ferroptosis, depending on the context. 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 2 parallel DNA repair pathways, one involving PARP and the other requiring BRCA1[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 (CTRP))[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 induction[5]. 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 therefore are 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 $GPX4^{high}$ ones[9]. Likewise, low expression of GPX4 (in concert with high expression of ALOX5) renders germinal centre B cell-like diffuse large B-cell lymphoma (DLBCL) 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 to select patients for cancer treatment with FINs that target other ferroptosis defence arms.

Ferroptosis in the 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 downregulating SLC7A11 expression, thereby augmenting lipid peroxidation and ferroptosis in tumours[28] (Fig. 3). Interestingly, IFNγ has also been shown to suppress SLC7A11-mediated cystine transport in macrophages[124], 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 cancer[28]. Of note, ferroptotic cancer cells can release several immunostimulatory signals, such as high mobility group box 1 (HMGB1), calreticulin, ATP and PE, 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, early ferroptotic cells (after 1- or 3-hours of treatment with a GPX4 inhibitor) by releasing immunostimulatory signals can promote the phenotypic maturation of dendritic cells and elicit a vaccination-like effect to activate antitumour immunity[127]. 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 immunity[130]. Similarly, myeloid-derived suppressor cells (MDSCs) with immunosuppressive functions exhibit resistance to ferroptosis that is driven by N-acylsphingosine amidohydrolase 2 (ASAH2)-mediated inhibition of the p53–heme oxygenase 1 (HMOX1) axis[131]; consequently, targeting ASAH2 to induce ferroptosis in MDSCs increases the activation of tumour-infiltrating cytotoxic $CD8^+$ T cells and promotes tumour suppression[131]. In addition, tumour-associated macrophages (TAMs) predominantly display the M2-like phenotype to suppress antitumour immunity[132]. 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 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 immunotherapy[133].

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 ferroptosis[134]. Furthermore, substantial lipid peroxides are detected in $CD8^+$ T cells derived from tumours, but not those from lymph nodes, suggesting that ferroptosis may be a metabolic vulnerability of tumour-specific $CD8^+$ T cells[135]. 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 immunity[136]. 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 function[137] (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 cells[138], which may explain the synergistic augmentation of antitumour immunity by targeting SLC7A11 in combination with ICIs[28]. 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 FINs[30]. Furthermore, growing evidence suggests that ferroptosis at least partly mediates the tumour-suppressive effects of several conventional cancer therapies, including RT[25,141–143], chemotherapy[26], targeted therapy[27,144] and immunotherapy[28], 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 (see 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[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, cystathionine β-synthase (CBS), and glycine N-methyltransferase (GNMT), can potentiate the susceptibility of tumours to FINs that block SLC7A11[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[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*- or *PTEN*-mutant xenograft tumours[17]. 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 (i.e., 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 mechanisms[151]. In response to RT, cancer cells evolve adaptive responses, such as upregulating SLC7A11 or GPX4 expression, to antagonize RT-induced ferroptosis[25]. Consequently, FINs targeting either SLC7A11 or GPX4 in combination with RT can radiosensitise 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 ferroptosis[152]. Likewise, FINs targeting SLC7A11 can sensitise cancer cells to chemotherapy (e.g., cisplatin and doxorubicin)[153,154], immunotherapy (e.g., ICIs)[28], and immunotherapy in combination with RT[143].

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[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-programmed cell death protein 1 (PD1) or programmed cell death 1 ligand 1 (PDL1) therapy, while metallothionein-1G (MT1G) induction renders cancer cells resistant to the multikinase 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 combining anti-PD1 or 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 have 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 cells[142] 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 (e.g., NCT04205357[161], NCT04092647[162], NCT02559778[163], NCT03247088[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 translation[165]. 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 mechanism will continue to provide key insights into targeting ferroptosis in cancer. The recently proposed compartmentalization model[9] suggests that there might exist additional ferroptosis defence systems localized in other organelles. Another key question in ferroptosis research is whether lipid peroxidation on the plasma membrane represents the final step or 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 in DHODH/GPX4 double inactivated cells[9] indicates additional ferroptosis execution mechanisms downstream of lipid peroxidation on the plasma membrane. Understanding these fundamental questions might 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, cell death executioner proteins involved in other cell death mechanisms, such as caspase, MLKL, and gasdermin D, 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 2 (PKCβII), which then phosphorylates ACSL4 and promotes ACSL4-mediated PUFA-PL synthesis and subsequent ferroptosis, thereby forming a positive feedforward loop to amplify ferroptosis[168]. Attenuation of the PKCβII-ACSL4 signalling axis dampens the efficacy of immunotherapy by suppressing tumour ferroptosis[168]. This study will inspire future investigations aimed to 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.

Acknowledgements

We thank Amy Ninetto from the Research Medical Library at MD Anderson for editing the manuscript, and apologize to colleagues whose work cannot be cited in this manuscript due to space limitations. B.G. is supported by The University of Texas MD Anderson Cancer Center, Emerson Collective Cancer Research Fund, and grants R01CA181196, R01CA244144 and R01CA247992 from the National Institutes of Health. The research from the authors' lab has also been supported by the National Institutes of Health Cancer Center Support Grant P30CA016672 to The University of Texas MD Anderson Cancer Center.

Glossary

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.

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 nonenzymatic reaction of labile iron and hydrogen peroxide (H_2O_2) 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.

GPX (Glutathione peroxidase). A family of peroxidases that use reduced glutathione as their cofactor to reduce hydroperoxide species to their corresponding alcohols.

System x_c^- An antiporter that imports cystine and exports glutamate; it consists of 2 subunits, including the transporter subunit SLC7A11 and the regulatory subunit SLC3A2.

Ubiquinone (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 ($CoQH_2$). 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 $CoQH_2$

Hypercholesterolemia 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 Molecular ensembles of iron and sulfur that function as protein co-factors to regulate iron homeostasis and redox reactions in response to oxidative stress.

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.
Cyst(e)inase	Engineered enzymes that degrade extracellular cysteine and cystine.
Transsulfuration pathway	A metabolic pathway that transfers sulphur from homocysteine to cysteine, leading to cysteine biosynthesis.

References

1. Dixon SJ et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 149, 1060–1072 (2012). This paper describe a new form of non-apoptotic cell death induced by the small molecule erastin, termed ferroptosis. [PubMed: 22632970]
2. Stockwell BR et al. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell 171, 273–285 (2017). [PubMed: 28985560]
3. Jiang X, Stockwell BR & Conrad M Ferroptosis: mechanisms, biology and role in disease. Nature Reviews Molecular Cell Biology 22, 266–282 (2021). [PubMed: 33495651]
4. Yang WS et al. Regulation of ferroptotic cancer cell death by GPX4. Cell 156, 317–331 (2014). This paper reports that GPX4 is a cardinal regulator of ferroptosis and that the small molecule RSL3 induces ferroptosis by directly inhibiting GPX4. [PubMed: 24439385]
5. Bersuker K et al. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. Nature 575, 688–692 (2019). [PubMed: 31634900]
6. Doll S et al. FSP1 is a glutathione-independent ferroptosis suppressor. Nature 575, 693–698 (2019). Along with Bersuker et al. (2019), this paper reports a GPX4-independent ferroptosis defence mechanism in plasma membrane involving NADPH, FSP1 and ubiquinone. [PubMed: 31634899]
7. Kraft VA et al. GTP cyclohydrolase 1/tetrahydrobiopterin counteract ferroptosis through lipid remodeling. ACS Central Science 6, 41–53 (2019). Together with Soula et al. (2020), this paper reports a GPX4-independent ferroptosis defence mechanism involving GCH1 and BH4. [PubMed: 31989025]
8. Soula M et al. Metabolic determinants of cancer cell sensitivity to canonical ferroptosis inducers. Nature chemical biology 16, 1351–1360 (2020). [PubMed: 32778843]
9. Mao C et al. DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. Nature 593, 586–590 (2021). This paper reports a targetable GPX4-independent ferroptosis defence mechanism in mitochondria involving NADPH, DHODH and ubiquinone. [PubMed: 33981038]
10. Ingold I et al. Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. Cell 172, 409–422. e421 (2018). [PubMed: 29290465]
11. Galluzzi L et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death & Differentiation 25, 486–541 (2018). [PubMed: 29362479]
12. Wiernicki B et al. Excessive phospholipid peroxidation distinguishes ferroptosis from other cell death modes including pyroptosis. Cell Death & Disease 11, 922 (2020). [PubMed: 33110056]
13. Kagan VE et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. Nature chemical biology 13, 81–90 (2017). This paper identifies the specific relevance of phosphatidylethanolamine phospholipids to ferroptosis. [PubMed: 27842066]
14. Hassannia B, Vandenabeele P & Berghe TV Targeting ferroptosis to iron out cancer. Cancer cell 35, 830–849 (2019). [PubMed: 31105042]
15. Jiang L et al. Ferroptosis as a p53-mediated activity during tumour suppression. Nature 520, 57–62 (2015). This study identified a new pattern of tumor suppression based on p53 regulation of cystine metabolism, ROS response and ferroptosis. [PubMed: 25799988]

16. Zhang Y et al. BAP1 links metabolic regulation of ferroptosis to tumour suppression. Nature cell biology 20, 1181–1192 (2018). This paper reports that the tumour suppressor BAP1 acts by sensitizing cells to ferroptosis by mitigating SLC7A11 expression. [PubMed: 30202049]
17. Yi J, Zhu J, Wu J, Thompson CB & Jiang X Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis via SREBP-mediated lipogenesis. Proceedings of the National Academy of Sciences 117, 31189–31197 (2020).
18. Ubellacker JM et al. Lymph protects metastasizing melanoma cells from ferroptosis. Nature 585, 113–118 (2020). This paper reports that melanoma cells exposed to the lymphatic environment can escape from ferroptosis and increases their survival during subsequent metastasis through the blood. [PubMed: 32814895]
19. Angeli JPF, Krysko DV & Conrad M Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. Nature Reviews Cancer 19, 405–414 (2019). [PubMed: 31101865]
20. Wu J et al. Intercellular interaction dictates cancer cell ferroptosis via NF2–YAP signalling. Nature 572, 402–406 (2019). This paper reports that cell-to-cell contacts confer resistance to ferroptosis via the NF2–Hippo–YAP axis and reveals several malignant mutations in this axis as a ferroptosis vulnerability. [PubMed: 31341276]
21. Hangauer MJ et al. Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. Nature 551, 247–250 (2017). This study reports that drug-resistant persist cancer cells after targeted therapy are hypersensitive to GPX4 inhibition. [PubMed: 29088702]
22. Viswanathan VS et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature 547, 453–457 (2017). This paper reports that drug-resistant and metastasis-prone mesenchymal carcinoma cells are vulnerable to ferroptosis. [PubMed: 28678785]
23. Zou Y et al. Plasticity of ether lipids promotes ferroptosis susceptibility and evasion. Nature 585, 603–608 (2020). This paper identifies PUFA-containing ether PLs (PUFA-ePLs) as additional substrates for lipid peroxidation and reports the peroxisome–ether-phospholipid axis-mediated ferroptosis vulnerability. [PubMed: 32939090]
24. Zou Y et al. A GPX4-dependent cancer cell state underlies the clear-cell morphology and confers sensitivity to ferroptosis. Nature Communications 10, 1617 (2019).
25. Lei G et al. The role of ferroptosis in ionizing radiation-induced cell death and tumor suppression. Cell research 30, 146–162 (2020). Together with Lang et al. (2019) and Ye (2020), this study reports that ionizing radiation induces ferroptosis and that ionizing radiation synergizes with FINs, and suggests that clinically used radiotherapy works, at least partially, by inducing ferroptosis. [PubMed: 31949285]
26. Guo J et al. Ferroptosis: a novel anti-tumor action for cisplatin. Cancer research and treatment: official journal of Korean Cancer Association 50, 445 (2018).
27. Sun X et al. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. Hepatology 63, 173–184 (2016). [PubMed: 26403645]
28. Wang W et al. CD8+ T cells regulate tumour ferroptosis during cancer immunotherapy. Nature 569, 270–274 (2019). This paper reports that activated CD8+ T cells promote ferroptosis in tumours through inhibition of SLC7A11, and that combination of immune checkpoint inhibitors with FINs is a promising strategy cancer therapy. [PubMed: 31043744]
29. Zhang Y et al. Imidazole ketone erastin induces ferroptosis and slows tumor growth in a mouse lymphoma model. Cell chemical biology 26, 623–633. e629 (2019). [PubMed: 30799221]
30. Liang C, Zhang X, Yang M & Dong X Recent progress in ferroptosis inducers for cancer therapy. Advanced materials 31, 1904197 (2019).
31. Gan B Mitochondrial regulation of ferroptosis. Journal of Cell Biology 220, e202105043 (2021). [PubMed: 34328510]
32. Dixon SJ et al. Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. ACS chemical biology 10, 1604–1609 (2015). [PubMed: 25965523]
33. Doll S et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. Nature chemical biology 13, 91–98 (2017). [PubMed: 27842070]
34. Lee H, Zhuang L & Gan B Energy stress inhibits ferroptosis via AMPK. Molecular & Cellular Oncology 7, 1761242 (2020). [PubMed: 32944623]

35. Li C et al. LKB1-AMPK axis negatively regulates ferroptosis by inhibiting fatty acid synthesis. Signal transduction and targeted therapy 5, 187 (2020). [PubMed: 32883948]
36. Shimada K et al. Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. Nature chemical biology 12, 497–503 (2016). [PubMed: 27159577]
37. Lee H et al. Energy-stress-mediated AMPK activation inhibits ferroptosis. Nature cell biology 22, 225–234 (2020). Together with Li et al. (2020), this paper reports that energy stress suppresses ferroptosis through activation of AMPK pathway. [PubMed: 32029897]
38. Chu B et al. ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway. Nature cell biology 21, 579–591 (2019). [PubMed: 30962574]
39. Cui W, Liu D, Gu W & Chu B Peroxisome-driven ether-linked phospholipids biosynthesis is essential for ferroptosis. Cell Death & Differentiation 28, 2536–2551 (2021). [PubMed: 33731874]
40. Lee H, Zhuang L & Gan B Ether phospholipids govern ferroptosis. Journal of Genetics and Genomics 48, 517–519 (2021). [PubMed: 34167916]
41. Conrad M & Pratt DA The chemical basis of ferroptosis. Nature chemical biology 15, 1137–1147 (2019). [PubMed: 31740834]
42. Shah R, Shchepinov MS & Pratt DA Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. ACS central science 4, 387–396 (2018). [PubMed: 29632885]
43. Gaschler MM & Stockwell BR Lipid peroxidation in cell death. Biochemical and biophysical research communications 482, 419–425 (2017). [PubMed: 28212725]
44. Yang WS et al. Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. Proceedings of the National Academy of Sciences 113, E4966–E4975 (2016).
45. Koppula P, Zhuang L & Gan B Cytochrome P450 reductase (POR) as a ferroptosis fuel. Protein & Cell 12, 675–679 (2021). [PubMed: 33539003]
46. Yan B et al. Membrane Damage during Ferroptosis Is Caused by Oxidation of Phospholipids Catalyzed by the Oxidoreductases POR and CYB5R1. Molecular Cell 81, 355–369. e310 (2021). [PubMed: 33321093]
47. Zou Y et al. Cytochrome P450 oxidoreductase contributes to phospholipid peroxidation in ferroptosis. Nature Chemical Biology 16, 302–309 (2020). [PubMed: 32080622]
48. Wenzel SE et al. PEBP1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. Cell 171, 628–641. e626 (2017). [PubMed: 29053969]
49. Angeli JPF et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. Nature cell biology 16, 1180–1191 (2014). [PubMed: 25402683]
50. Magtanong L et al. Exogenous monounsaturated fatty acids promote a ferroptosis-resistant cell state. Cell chemical biology 26, 420–432. e429 (2019). [PubMed: 30686757]
51. Paton CM & Ntambi JM Biochemical and physiological function of stearoyl-CoA desaturase. American Journal of Physiology-Endocrinology and Metabolism 297, E28–E37 (2009). [PubMed: 19066317]
52. Tesfay L et al. Stearoyl-coa desaturase 1 protects ovarian cancer cells from ferroptotic cell death. Cancer research 79, 5355–5366 (2019). [PubMed: 31270077]
53. Chen X, Yu C, Kang R & Tang D Iron metabolism in ferroptosis. Frontiers in Cell and Developmental Biology 8, 590226 (2020). [PubMed: 33117818]
54. Gao M et al. Ferroptosis is an autophagic cell death process. Cell research 26, 1021–1032 (2016). [PubMed: 27514700]
55. Hou W et al. Autophagy promotes ferroptosis by degradation of ferritin. Autophagy 12, 1425–1428 (2016). [PubMed: 27245739]
56. Kremer DM et al. GOT1 inhibition promotes pancreatic cancer cell death by ferroptosis. Nature communications 12, 4860 (2021).
57. Murphy MP How mitochondria produce reactive oxygen species. Biochemical journal 417, 1–13 (2009). [PubMed: 19061483]
58. Zheng J & Conrad M The Metabolic Underpinnings of Ferroptosis. Cell Metabolism 32, 920–937 (2020). [PubMed: 33217331]
59. Friedman JR & Nunnari J Mitochondrial form and function. Nature 505, 335–343 (2014). [PubMed: 24429632]

60. Vasan K, Werner M & Chandel NS Mitochondrial metabolism as a target for cancer therapy. Cell metabolism 32, 341–352 (2020). [PubMed: 32668195]
61. Gao M, Monian P, Quadri N, Ramasamy R & Jiang X Glutaminolysis and transferrin regulate ferroptosis. Molecular cell 59, 298–308 (2015). [PubMed: 26166707]
62. Gao M et al. Role of mitochondria in ferroptosis. Molecular cell 73, 354–363. e353 (2019). [PubMed: 30581146]
63. Heldt H & Piechulla B 15-Lipids are membrane constituents and function as carbon stores. Plant Biochemistry, 359–398 (2011).
64. Brigelius-Flohé R & Maiorino M Glutathione peroxidases. Biochimica et Biophysica Acta (BBA)-General Subjects 1830, 3289–3303 (2013). [PubMed: 23201771]
65. Brigelius-Flohé R & Flohé L Regulatory phenomena in the glutathione peroxidase superfamily. Antioxidants & redox signaling 33, 498–516 (2020). [PubMed: 31822117]
66. Seibt TM, Proneth B & Conrad M Role of GPX4 in ferroptosis and its pharmacological implication. Free Radical Biology and Medicine 133, 144–152 (2019). [PubMed: 30219704]
67. Ursini F, Maiorino M, Valente M, Ferri L & Gregolin C Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism 710, 197–211 (1982). [PubMed: 7066358]
68. Pushpa-Rekha TR, Burdsall AL, Oleksa LM, Chisolm GM & Driscoll DM Rat phospholipid-hydroperoxide glutathione peroxidase: cDNA cloning and identification of multiple transcription and translation start sites. Journal of Biological Chemistry 270, 26993–26999 (1995). [PubMed: 7592947]
69. Pfeifer H et al. Identification of a specific sperm nuclei selenoenzyme necessary for protamine thiol cross-linking during sperm maturation. The FASEB Journal 15, 1236–1238 (2001). [PubMed: 11344099]
70. Maiorino M et al. Distinct promoters determine alternative transcription of gpx-4 into phospholipid-hydroperoxide glutathione peroxidase variants. Journal of Biological Chemistry 278, 34286–34290 (2003). [PubMed: 12819198]
71. Moreno SG, Laux G, Brielmeier M, Bornkamm GW & Conrad M Testis-specific expression of the nuclear form of phospholipid hydroperoxide glutathione peroxidase (PHGPx). Biological Chemistry 384, 635–643 (2003). [PubMed: 12751792]
72. Yant LJ et al. The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. Free Radical Biology and Medicine 34, 496–502 (2003). [PubMed: 12566075]
73. Conrad M et al. The nuclear form of phospholipid hydroperoxide glutathione peroxidase is a protein thiol peroxidase contributing to sperm chromatin stability. Molecular and cellular biology 25, 7637–7644 (2005). [PubMed: 16107710]
74. Liang H et al. Short form glutathione peroxidase 4 is the essential isoform required for survival and somatic mitochondrial functions. Journal of Biological Chemistry 284, 30836–30844 (2009). [PubMed: 19744930]
75. Schneider M et al. Mitochondrial glutathione peroxidase 4 disruption causes male infertility. The FASEB journal 23, 3233–3242 (2009). [PubMed: 19417079]
76. Imai H et al. Depletion of selenoprotein GPx4 in spermatocytes causes male infertility in mice. Journal of Biological Chemistry 284, 32522–32532 (2009). [PubMed: 19783653]
77. Forman HJ, Zhang H & Rinna A Glutathione: overview of its protective roles, measurement, and biosynthesis. Molecular aspects of medicine 30, 1–12 (2009). [PubMed: 18796312]
78. Aquilano K, Baldelli S & Ciriolo MR Glutathione: new roles in redox signaling for an old antioxidant. Frontiers in pharmacology 5, 196 (2014). [PubMed: 25206336]
79. Koppula P, Zhang Y, Zhuang L & Gan B Amino acid transporter SLC7A11/xCT at the crossroads of regulating redox homeostasis and nutrient dependency of cancer. Cancer Communications 38, 12 (2018). [PubMed: 29764521]
80. Liu X et al. Cystine transporter regulation of pentose phosphate pathway dependency and disulfide stress exposes a targetable metabolic vulnerability in cancer. Nature cell biology 22, 476–486 (2020). [PubMed: 32231310]

81. Sato H, Tamba M, Ishii T & Bannai S Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. Journal of Biological Chemistry 274, 11455–11458 (1999). [PubMed: 10206947]
82. Koppula P, Zhuang L & Gan B Cystine transporter SLC7A11/xCT in cancer: ferroptosis, nutrient dependency, and cancer therapy. Protein & cell 12, 599–620 (2021). [PubMed: 33000412]
83. Stefely JA & Pagliarini DJ Biochemistry of mitochondrial coenzyme Q biosynthesis. Trends in biochemical sciences 42, 824–843, doi:10.1016/j.tibs.2017.06.008 (2017). [PubMed: 28927698]
84. Crane FL Discovery of ubiquinone (coenzyme Q) and an overview of function. Mitochondrion 7, S2–S7 (2007). [PubMed: 17446142]
85. Kalen A, Norling B, Appelkvist EL & Dallner G Ubiquinone biosynthesis by the microsomal fraction from rat liver. Biochim Biophys Acta 926, 70–78, doi:10.1016/0304-4165(87)90183-8 (1987). [PubMed: 3651503]
86. Turunen M, Olsson J & Dallner G Metabolism and function of coenzyme Q. Biochim Biophys Acta 1660, 171–199, doi:10.1016/j.bbamem.2003.11.012 (2004). [PubMed: 14757233]
87. Takahashi T, Okamoto T, Mori K, Sayo H & Kishi T Distribution of ubiquinone and ubiquinol homologues in rat tissues and subcellular fractions. Lipids 28, 803–809, doi:10.1007/BF02536234 (1993). [PubMed: 8231656]
88. Morre DJ & Morre DM Non-mitochondrial coenzyme Q. Biofactors 37, 355–360, doi:10.1002/biof.156 (2011). [PubMed: 21674641]
89. Thöny B, Auerbach G & Blau N Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochemical Journal 347, 1–16 (2000). [PubMed: 10727395]
90. Chen D et al. iPLA2β-mediated lipid detoxification controls p53-driven ferroptosis independent of GPX4. Nature Communications 12, 3644 (2021).
91. Sun W-Y et al. Phospholipase iPLA 2 β averts ferroptosis by eliminating a redox lipid death signal. Nature Chemical Biology 17, 465–476 (2021). [PubMed: 33542532]
92. Tan SK et al. Obesity-dependent adipokine chemerin suppresses fatty acid oxidation to confer ferroptosis resistance. Cancer discovery 11, 2072–2093 (2021). [PubMed: 33757970]
93. Liu W et al. Dysregulated cholesterol homeostasis results in resistance to ferroptosis increasing tumorigenicity and metastasis in cancer. Nature communications 12, 5103 (2021).
94. Sviderskiy VO, Terzi EM & Possemato R in Ferroptosis in Health and Disease 215–237 (Springer, 2019).
95. Alvarez SW et al. NFS1 undergoes positive selection in lung tumours and protects cells from ferroptosis. Nature 551, 639–643 (2017). [PubMed: 29168506]
96. Du J et al. Identification of Frataxin as a regulator of ferroptosis. Redox biology 32, 101483 (2020). [PubMed: 32169822]
97. Kim EH, Shin D, Lee J, Jung AR & Roh J-L CISD2 inhibition overcomes resistance to sulfasalazine-induced ferroptotic cell death in head and neck cancer. Cancer letters 432, 180–190 (2018). [PubMed: 29928961]
98. Brown CW et al. Prominin2 drives ferroptosis resistance by stimulating iron export. Developmental Cell 51, 575–586. e574 (2019). [PubMed: 31735663]
99. Anandhan A, Dodson M, Schmidlin CJ, Liu P & Zhang DD Breakdown of an ironclad defense system: the critical role of NRF2 in mediating ferroptosis. Cell chemical biology 27, 436–447 (2020). [PubMed: 32275864]
100. Chen D et al. NRF2 is a major target of ARF in p53-independent tumor suppression. Molecular cell 68, 224–232. e224 (2017). [PubMed: 28985506]
101. Dodson M, Castro-Portuguez R & Zhang DD NRF2 plays a critical role in mitigating lipid peroxidation and ferroptosis. Redox biology 23, 101107 (2019). [PubMed: 30692038]
102. Hu K et al. Suppression of the SLC7A11/glutathione axis causes synthetic lethality in KRAS-mutant lung adenocarcinoma. The Journal of Clinical Investigation 130, 1752–1766 (2020). [PubMed: 31874110]
103. Lim JK et al. Cystine/glutamate antiporter xCT (SLC7A11) facilitates oncogenic RAS transformation by preserving intracellular redox balance. Proceedings of the National Academy of Sciences 116, 9433–9442 (2019).

104. de la Vega MR, Chapman E & Zhang DD NRF2 and the hallmarks of cancer. Cancer cell 34, 21–43 (2018). [PubMed: 29731393]
105. Garcia-Bermudez J et al. Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death. Nature 567, 118–122 (2019). [PubMed: 30760928]
106. Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F & Lisanti MP Cancer metabolism: a therapeutic perspective. Nature reviews Clinical oncology 14, 11–31 (2017).
107. Wolpaw AJ & Dang CV Exploiting metabolic vulnerabilities of cancer with precision and accuracy. Trends in cell biology 28, 201–212 (2018). [PubMed: 29229182]
108. Tsoi J et al. Multi-stage differentiation defines melanoma subtypes with differential vulnerability to drug-induced iron-dependent oxidative stress. Cancer cell 33, 890–904. e895 (2018). [PubMed: 29657129]
109. Krebs AM et al. The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. Nature cell biology 19, 518–529 (2017). [PubMed: 28414315]
110. Lin C-C et al. DDR2 upregulation confers ferroptosis susceptibility of recurrent breast tumors through the Hippo pathway. Oncogene 40, 2018–2034 (2021). [PubMed: 33603168]
111. Lee J-Y et al. Polyunsaturated fatty acid biosynthesis pathway determines ferroptosis sensitivity in gastric cancer. Proceedings of the National Academy of Sciences 117, 32433–32442 (2020).
112. Müller S et al. CD44 regulates epigenetic plasticity by mediating iron endocytosis. Nature Chemistry 12, 929–938 (2020).
113. Bebber CM et al. Ferroptosis response segregates small cell lung cancer (SCLC) neuroendocrine subtypes. Nature communications 12, 2048 (2021).
114. Verma N et al. Synthetic lethal combination targeting BET uncovered intrinsic susceptibility of TNBC to ferroptosis. Science advances 6, eaba8968 (2020). [PubMed: 32937365]
115. Yang W-H et al. The hippo pathway effector TAZ regulates ferroptosis in renal cell carcinoma. Cell reports 28, 2501–2508. e2504 (2019). [PubMed: 31484063]
116. Iliopoulos O, Kibel A, Gray S & Kaelin WG Tumour suppression by the human von Hippel-Lindau gene product. Nature medicine 1, 822–826 (1995).
117. Kaelin WG Jr The von Hippel–Lindau tumour suppressor protein: O 2 sensing and cancer. Nature Reviews Cancer 8, 865–873 (2008). [PubMed: 18923434]
118. Miess H et al. The glutathione redox system is essential to prevent ferroptosis caused by impaired lipid metabolism in clear cell renal cell carcinoma. Oncogene 37, 5435–5450 (2018). [PubMed: 29872221]
119. Poursaitidis I et al. Oncogene-selective sensitivity to synchronous cell death following modulation of the amino acid nutrient cystine. Cell reports 18, 2547–2556 (2017). [PubMed: 28297659]
120. Zhang Y et al. mTORC1 couples cyst (e) ine availability with GPX4 protein synthesis and ferroptosis regulation. Nature Communications 12, 1589 (2021). This study uncovers a ferroptosis modulatory mechanism that coordinates GPX4 protein synthesis with cyst(e)ine availability and suggests the combination of mTORC1 inhibitors with FINs for cancer therapy.
121. Wang T-X et al. The oncometabolite 2-hydroxyglutarate produced by mutant IDH1 sensitizes cells to ferroptosis. Cell death & disease 10, 755 (2019). [PubMed: 31591388]
122. Kaelin WG The concept of synthetic lethality in the context of anticancer therapy. Nature reviews cancer 5, 689–698 (2005). [PubMed: 16110319]
123. Schmitt A et al. Dimethyl fumarate induces ferroptosis and impairs NF-κB/STAT3 signaling in DLBCL. Blood, The Journal of the American Society of Hematology 138, 871–884 (2021).
124. Sato H, Fujiwara K, Sagara J. i. & Bannai S Induction of cystine transport activity in mouse peritoneal macrophages by bacterial lipopolysaccharide. Biochemical Journal 310, 547–551 (1995). [PubMed: 7654193]
125. Wen Q, Liu J, Kang R, Zhou B & Tang D The release and activity of HMGB1 in ferroptosis. Biochemical and biophysical research communications 510, 278–283 (2019). [PubMed: 30686534]
126. Luo X et al. Oxygenated phosphatidylethanolamine navigates phagocytosis of ferroptotic cells by interacting with TLR2. Cell Death & Differentiation 28, 1971–1989 (2021). [PubMed: 33432112]

127. Efimova I et al. Vaccination with early ferroptotic cancer cells induces efficient antitumor immunity. Journal for immunotherapy of cancer 8, e001369 (2020). [PubMed: 33188036]
128. Yu B, Choi B, Li W & Kim D-H Magnetic field boosted ferroptosis-like cell death and responsive MRI using hybrid vesicles for cancer immunotherapy. Nature communications 11, 3637 (2020).
129. Li C, Jiang P, Wei S, Xu X & Wang J Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. Molecular cancer 19, 1–23 (2020). [PubMed: 31901224]
130. Xu C et al. The glutathione peroxidase Gpx4 prevents lipid peroxidation and ferroptosis to sustain Treg cell activation and suppression of antitumor immunity. Cell Reports 35, 109235 (2021). [PubMed: 34133924]
131. Zhu H et al. Asah2 Represses the p53–Hmox1 Axis to Protect Myeloid-Derived Suppressor Cells from Ferroptosis. The Journal of Immunology 206, 1395–1404 (2021). [PubMed: 33547170]
132. Xia Y et al. Engineering macrophages for cancer immunotherapy and drug delivery. Advanced Materials 32, 2002054 (2020).
133. Kapralov AA et al. Redox lipid reprogramming commands susceptibility of macrophages and microglia to ferroptotic death. Nature chemical biology 16, 278–290 (2020). [PubMed: 32080625]
134. Matsushita M et al. T cell lipid peroxidation induces ferroptosis and prevents immunity to infection. Journal of Experimental Medicine 212, 555–568 (2015). [PubMed: 25824823]
135. Drijvers JM et al. Pharmacologic Screening Identifies Metabolic Vulnerabilities of CD8+ T Cells. Cancer immunology research 9, 184–199 (2020). [PubMed: 33277233]
136. Ma X et al. CD36-mediated ferroptosis dampens intratumoral CD8+ T cell effector function and impairs their antitumor ability. Cell metabolism 33, 1001–1012.e1005. (2021). [PubMed: 33691090]
137. Yao Y et al. Selenium–GPX4 axis protects follicular helper T cells from ferroptosis. Nature Immunology 22, 1127–1139 (2021). [PubMed: 34413521]
138. Arensman MD et al. Cystine–glutamate antiporter xCT deficiency suppresses tumor growth while preserving antitumor immunity. Proceedings of the National Academy of Sciences 116, 9533–9542 (2019).
139. GMÜNDER H, ECK HP & DRÖGE W Low membrane transport activity for cystine in resting and mitogenically stimulated human lymphocyte preparations and human T cell clones. European journal of biochemistry 201, 113–117 (1991). [PubMed: 1680678]
140. Pacheco R et al. Glutamate released by dendritic cells as a novel modulator of T cell activation. The Journal of Immunology 177, 6695–6704 (2006). [PubMed: 17082582]
141. Ye LF et al. Radiation-induced lipid peroxidation triggers ferroptosis and synergizes with ferroptosis inducers. ACS chemical biology 15, 469–484 (2020). [PubMed: 31899616]
142. Lei G et al. Ferroptosis as a mechanism to mediate p53 function in tumor radiosensitivity. Oncogene 40, 3533–3547 (2021). This study identifies a previously unappreciated role of ferroptosis in p53-mediated radiosensitization and suggests the combination of radiotherapy and FINs for treatment of p53-mutant cancers. [PubMed: 33927351]
143. Lang X et al. Radiotherapy and immunotherapy promote tumoral lipid oxidation and ferroptosis via synergistic repression of SLC7A11. Cancer discovery 9, 1673–1685 (2019). [PubMed: 31554642]
144. Dixon SJ et al. Pharmacological inhibition of cystine–glutamate exchange induces endoplasmic reticulum stress and ferroptosis. Elife 3, e02523 (2014). [PubMed: 24844246]
145. Cao J et al. DJ-1 suppresses ferroptosis through preserving the activity of S-adenosyl homocysteine hydrolase. Nature communications 11, 1251 (2020).
146. Zhu J et al. Transsulfuration activity can support cell growth upon extracellular cysteine limitation. Cell metabolism 30, 865–876. e865 (2019). [PubMed: 31607565]
147. Liu N, Lin X & Huang C Activation of the reverse transsulfuration pathway through NRF2/CBS confers erastin-induced ferroptosis resistance. British journal of cancer 122, 279–292 (2020). [PubMed: 31819185]
148. Wang L et al. A pharmacological probe identifies cystathionine β-synthase as a new negative regulator for ferroptosis. Cell death & disease 9, 1025 (2018). [PubMed: 30287840]

149. Song X et al. PDK4 dictates metabolic resistance to ferroptosis by suppressing pyruvate oxidation and fatty acid synthesis. Cell Reports 34, 108767 (2021). [PubMed: 33626342]
150. Badgley MA et al. Cysteine depletion induces pancreatic tumor ferroptosis in mice. Science 368, 85–89 (2020). [PubMed: 32241947]
151. Lei G, Mao C, Yan Y, Zhuang L & Gan B Ferroptosis, radiotherapy, and combination therapeutic strategies. Protein & Cell 12, 836–857 (2021). [PubMed: 33891303]
152. Zhu S et al. HSPA5 regulates ferroptotic cell death in cancer cells. Cancer research 77, 2064–2077 (2017). [PubMed: 28130223]
153. Yu Y et al. The ferroptosis inducer erastin enhances sensitivity of acute myeloid leukemia cells to chemotherapeutic agents. Molecular & cellular oncology 2, e1054549 (2015). [PubMed: 27308510]
154. Yamaguchi H et al. Caspase-independent cell death is involved in the negative effect of EGF receptor inhibitors on cisplatin in non–small cell lung cancer cells. Clinical cancer research 19, 845–854 (2013). [PubMed: 23344263]
155. Pan X et al. Erastin decreases radioresistance of NSCLC cells partially by inducing GPX4−mediated ferroptosis. Oncology letters 17, 3001–3008 (2019). [PubMed: 30854078]
156. Xie L et al. Solute carrier protein family may involve in radiation-induced radioresistance of non-small cell lung cancer. Journal of cancer research and clinical oncology 137, 1739 (2011). [PubMed: 21909646]
157. Roh J-L, Kim EH, Jang HJ, Park JY & Shin D Induction of ferroptotic cell death for overcoming cisplatin resistance of head and neck cancer. Cancer letters 381, 96–103 (2016). [PubMed: 27477897]
158. Zhou H-H et al. Erastin reverses ABCB1-mediated docetaxel resistance in ovarian cancer. Frontiers in oncology 9, 1398 (2019). [PubMed: 31921655]
159. Jiang Z et al. TYRO3 induces anti–PD-1/PD-L1 therapy resistance by limiting innate immunity and tumoral ferroptosis. Journal of Clinical Investigation 131, e139434 (2021). [PubMed: 33855973]
160. Sun X et al. Metallothionein-1G facilitates sorafenib resistance through inhibition of ferroptosis. Hepatology 64, 488–500 (2016). [PubMed: 27015352]
161. US National Library of Medicine. ClinicalTrials.gov, https://ClinicalTrials.gov/show/NCT04205357 (2019).
162. US National Library of Medicine. ClinicalTrials.gov, https://ClinicalTrials.gov/show/NCT04092647 (2019).
163. US National Library of Medicine. ClinicalTrials.gov, https://ClinicalTrials.gov/show/NCT02559778 (2015).
164. US National Library of Medicine. ClinicalTrials.gov, https://ClinicalTrials.gov/show/NCT03247088 (2017).
165. Eaton JK et al. Selective covalent targeting of GPX4 using masked nitrile-oxide electrophiles. Nature chemical biology 16, 497–506 (2020). [PubMed: 32231343]
166. Hassannia B et al. Nano-targeted induction of dual ferroptotic mechanisms eradicates high-risk neuroblastoma. The Journal of clinical investigation 128, 3341–3355 (2018). [PubMed: 29939160]
167. Woo JH et al. Elucidating compound mechanism of action by network perturbation analysis. Cell 162, 441–451 (2015). [PubMed: 26186195]
168. Zhang H-L et al. PKCβII phosphorylates ACSL4 to amplify lipid peroxidation to induce ferroptosis. Nature Cell Biology, 88–89 (2022). [PubMed: 35027735]
169. Yang Y et al. Nedd4 ubiquitylates VDAC2/3 to suppress erastin-induced ferroptosis in melanoma. Nature communications 11, 433 (2020).
170. Feng H & Stockwell BR Unsolved mysteries: How does lipid peroxidation cause ferroptosis? PLoS biology 16, e2006203 (2018). [PubMed: 29795546]
171. Larraufie M-H et al. Incorporation of metabolically stable ketones into a small molecule probe to increase potency and water solubility. Bioorganic & medicinal chemistry letters 25, 4787–4792 (2015). [PubMed: 26231156]

172. Plosker GL & Croom KF Sulfasalazine. Drugs 65, 1825–1849 (2005). [PubMed: 16114981]
173. Gout P, Buckley A, Simms C & Bruchovsky N Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the xc− cystine transporter: a new action for an old drug. Leukemia 15, 1633–1640 (2001). [PubMed: 11587223]
174. Robert SM et al. SLC7A11 expression is associated with seizures and predicts poor survival in patients with malignant glioma. Science translational medicine 7, 289ra286–289ra286 (2015).
175. Zheng J et al. Sorafenib fails to trigger ferroptosis across a wide range of cancer cell lines. Cell Death & Disease 12, 698 (2021). [PubMed: 34257282]
176. Cramer SL et al. Systemic depletion of L-cyst (e) ine with cyst (e) inase increases reactive oxygen species and suppresses tumor growth. Nature medicine 23, 120–127 (2017).
177. Gaschler MM et al. FINO 2 initiates ferroptosis through GPX4 inactivation and iron oxidation. Nature chemical biology 14, 507–515 (2018). [PubMed: 29610484]
178. Bykov VJ, Eriksson SE, Bianchi J & Wiman KG Targeting mutant p53 for efficient cancer therapy. Nature Reviews Cancer 18, 89–102 (2018). [PubMed: 29242642]
179. Bieging KT, Mello SS & Attardi LD Unravelling mechanisms of p53-mediated tumour suppression. Nature Reviews Cancer 14, 359–370 (2014).
180. Wang Y et al. Epigenetic regulation of ferroptosis by H2B monoubiquitination and p53. EMBO reports 20, e47563 (2019). [PubMed: 31267712]
181. Ou Y, Wang S-J, Li D, Chu B & Gu W Activation of SAT1 engages polyamine metabolism with p53-mediated ferroptotic responses. Proceedings of the National Academy of Sciences 113, E6806–E6812 (2016).
182. Zhang Y et al. Ferredoxin reductase is critical for p53-dependent tumor suppression via iron regulatory protein 2. Genes & development 31, 1243–1256 (2017). [PubMed: 28747430]
183. Jennis M et al. An African-specific polymorphism in the TP53 gene impairs p53 tumor suppressor function in a mouse model. Genes & development 30, 918–930 (2016). [PubMed: 27034505]
184. Wang S-J et al. Acetylation is crucial for p53-mediated ferroptosis and tumor suppression. Cell reports 17, 366–373 (2016). [PubMed: 27705786]
185. Tarangelo A et al. p53 suppresses metabolic stress-induced ferroptosis in cancer cells. Cell reports 22, 569–575 (2018). [PubMed: 29346757]
186. Xie Y et al. The tumor suppressor p53 limits ferroptosis by blocking DPP4 activity. Cell reports 20, 1692–1704 (2017). [PubMed: 28813679]
187. Carbone M et al. BAP1 and cancer. Nature Reviews Cancer 13, 153–159 (2013).
188. Ventii KH et al. BRCA1-associated protein-1 is a tumor suppressor that requires deubiquitinating activity and nuclear localization. Cancer research 68, 6953–6962 (2008). [PubMed: 18757409]
189. Carbone M et al. Biological mechanisms and clinical significance of BAP1 mutations in human cancer. Cancer discovery 10, 1103–1120 (2020). [PubMed: 32690542]
190. Network CGAR Comprehensive genomic characterization of squamous cell lung cancers. Nature 489, 519 (2012). [PubMed: 22960745]
191. Collisson E et al. Comprehensive molecular profiling of lung adenocarcinoma: The cancer genome atlas research network. Nature 511, 543–550 (2014). [PubMed: 25079552]
192. Baird L & Yamamoto M The molecular mechanisms regulating the KEAP1-NRF2 pathway. Molecular and cellular biology 40, e00099–00020 (2020). [PubMed: 32284348]
193. Kensler TW, Wakabayashi N & Biswal S Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. Annu. Rev. Pharmacol. Toxicol 47, 89–116 (2007). [PubMed: 16968214]
194. Sasaki H et al. Electrophile response element-mediated induction of the cystine/glutamate exchange transporter gene expression. Journal of Biological Chemistry 277, 44765–44771 (2002). [PubMed: 12235164]
195. Sherr CJ Divorcing ARF and p53: an unsettled case. Nature Reviews Cancer 6, 663–673 (2006). [PubMed: 16915296]
196. Fruman DA et al. The PI3K pathway in human disease. Cell 170, 605–635 (2017). [PubMed: 28802037]

197. Zhang Y et al. A pan-cancer proteogenomic atlas of PI3K/AKT/mTOR pathway alterations. Cancer cell 31, 820–832. e823 (2017). [PubMed: 28528867]

198. Lei G, Zhuang L & Gan B mTORC1 and ferroptosis: Regulatory mechanisms and therapeutic potential. BioEssays, 2100093 (2021).

199. Liu Y, Wang Y, Liu J, Kang R & Tang D Interplay between MTOR and GPX4 signaling modulates autophagy-dependent ferroptotic cancer cell death. Cancer Gene Therapy 28, 55–63 (2021). [PubMed: 32457486]

200. Prior IA, Lewis PD & Mattos C A comprehensive survey of Ras mutations in cancer. Cancer research 72, 2457–2467 (2012). [PubMed: 22589270]

201. Pylayeva-Gupta Y, Grabocka E & Bar-Sagi D RAS oncogenes: weaving a tumorigenic web. Nature Reviews Cancer 11, 761–774 (2011). [PubMed: 21993244]

202. Padanad MS et al. Fatty acid oxidation mediated by Acyl-CoA synthetase long chain 3 is required for mutant KRAS lung tumorigenesis. Cell reports 16, 1614–1628 (2016). [PubMed: 27477280]

203. Wang X et al. NEDD4–1 is a proto-oncogenic ubiquitin ligase for PTEN. Cell 128, 129–139 (2007). [PubMed: 17218260]

204. Yagoda N et al. RAS–RAF–MEK-dependent oxidative cell death involving voltage-dependent anion channels. Nature 447, 865–869 (2007).

205. Cao J, Lou S, Ying M & Yang B DJ-1 as a human oncogene and potential therapeutic target. Biochemical pharmacology 93, 241–250 (2015). [PubMed: 25498803]

206. Leclerc D et al. Oncogenic role of PDK4 in human colon cancer cells. British journal of cancer 116, 930–936 (2017). [PubMed: 28208156]

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 action[170]. 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 solubility[170]. Its analogue imidazole ketone erastin (IKE) has improved potency, solubility, and metabolic stability[171], and has been shown to induce ferroptosis and suppress tumour growth *in vivo* [29]. Sulfasalazine, a US Food and Drug Administration (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 potency[170], its activity in patients with glioblastoma has been demonstrated[174] 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[144]; although it has been shown to induce ferroptosis, it also induces other nonferroptotic cellular effects[144,170], and its classification as a FIN was challenged by a recent study[175]. 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-nitroketoxime (known as JKE-1674) in cells and subsequently forms a nitrile-oxide electrophile, which covalently inhibits GPX4 with remarkable selectivity[165]. Notably, JKE-1674 exhibits improved pharmacokinetic properties[165], 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[36], act by depleting both GPX4 protein and ubiquinone. Class IV FINs, such as $FINO_2$[177], act by oxidizing iron and indirectly inactivating GPX4. However, these FINs have not been assessed *in vivo*.

Box 2.

Ferroptosis regulation by tumour suppressors

p53, the most frequently mutated tumour suppressor[178], 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 BRCA1-associated protein 1 (*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 carcinoma (RCC)[189]. 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 models[16]. In addition, the ability of BAP1 to restrain SLC7A11 expression and promote ferroptosis is compromised in cells with cancer-associated *BAP1* mutations[16].

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[99,101,194]. In addition, ARF is a well-established tumour suppressor that is critical for p53 activation in response to oncogenic stress[195]. Interestingly, ARF was recently shown to inhibit NRF2 transcriptional activity independently of KEAP1 or p53[100]. In *ARF*-mutated tumours, NRF2 and its downstream target genes (such as *SLC7A11*) are activated, enabling cancer cells to evade ferroptosis under oxidative stress[100]. 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.

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 ferroptosis[17]. Oncogenic activation of the PI3K signalling pathway promotes the activation of mTOR complex 1 (mTORC1)[197], which inhibits ferroptosis through at least 3 parallel mechanisms[198]: promoting glutathione peroxidase 4 (GPX4) protein synthesis[120], suppressing autophagy-dependent ferroptosis[199] and boosting monounsaturated fatty acid-containing phospholipid (MUFA-PL) synthesis by increasing stearoyl coenzyme A desaturase 1 (SCD1) expression[17]. 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) upregulation[103]. Furthermore, pharmacological or genetic inhibition of SLC7A11 severely impairs mutant KRAS-induced tumour growth, suggesting that SLC7A11 is required for mutant KRAS-mediated tumourigenesis[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 reprogram 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 noncanonical oncogenes also contribute to the escape of cancer cells from ferroptosis. Neural precursor cell expressed, developmentally down-regulated 4 (NEDD4), a ubiquitin ligase with oncogenic functions[203], can be induced by the ferroptosis inducer erastin[169]. Erastin-induced ferroptosis requires voltage-dependent anion channel 2/3 (VDAC2/3)[204]; interestingly, VDAC2/3 expression was reduced in a NEDD4-dependent manner upon erastin treatment, thereby restricting erastin-induced ferroptosis[169]. Other proteins with oncogenic activities, such as DJ1[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, 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.

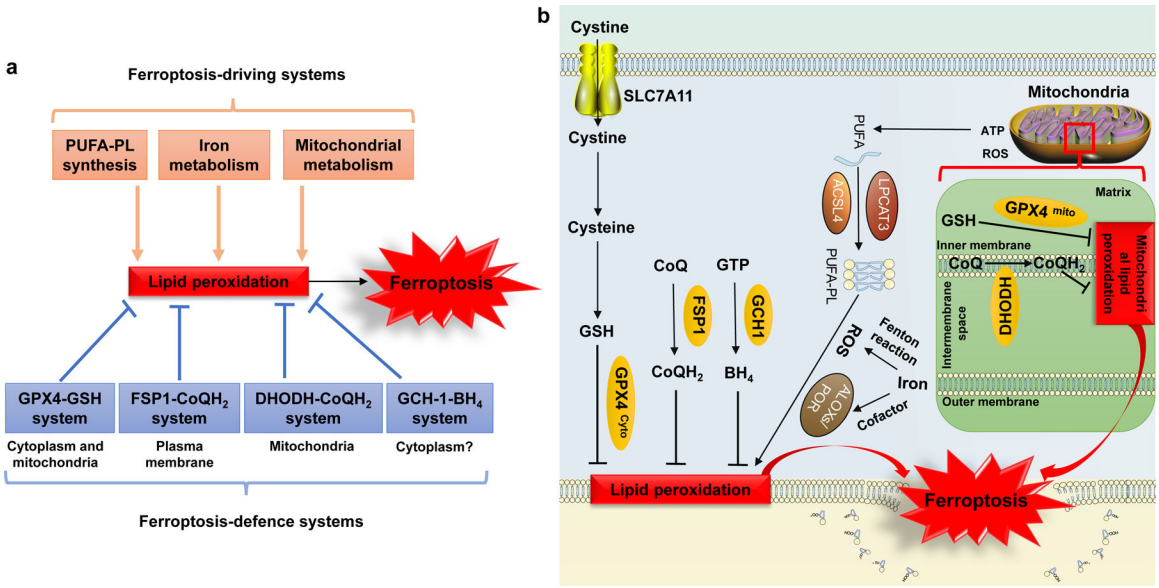

Figure 1. Ferroptosis-driving and -defence mechanisms.

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 ($CoQH_2$) system, the dihydroorotate dehydrogenase (DHODH)–$CoQH_2$ system, and the GTP cyclohydroxylase-1 (GCH1)– tetrahydrobiopterin (BH_4) 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 nonenzymatic and enzymatic mechanisms. Iron initiates the nonenzymatic 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 4 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 ($GPX4^{mito}$) with DHODH in the mitochondria to neutralize lipid peroxides. The subcellular compartment in which the GCH1–BH_4 system operates remains to be defined. CoQ, coenzyme Q (also known as ubiquinone).

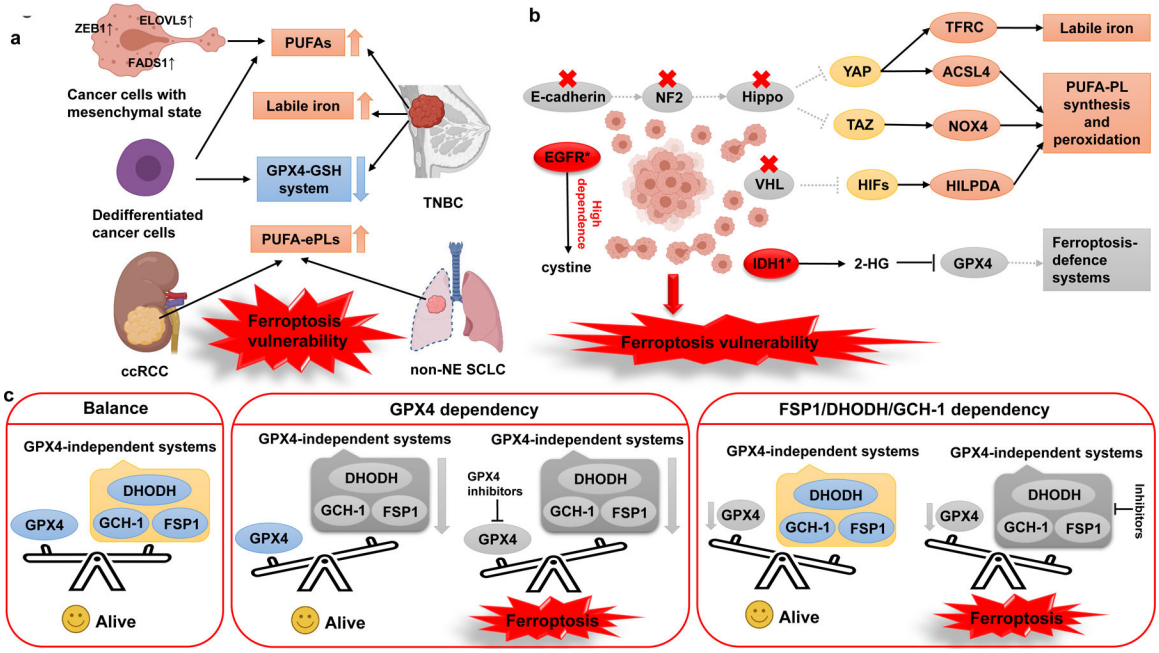

Figure 2. Ferroptosis as a vulnerability in cancer.

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)- 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 Von Hippel-Lindau (*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 (NSCLCs) 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. * indicates either a mutation or loss of the gene, dependent on the gene.

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 cyclohydroxylase-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.

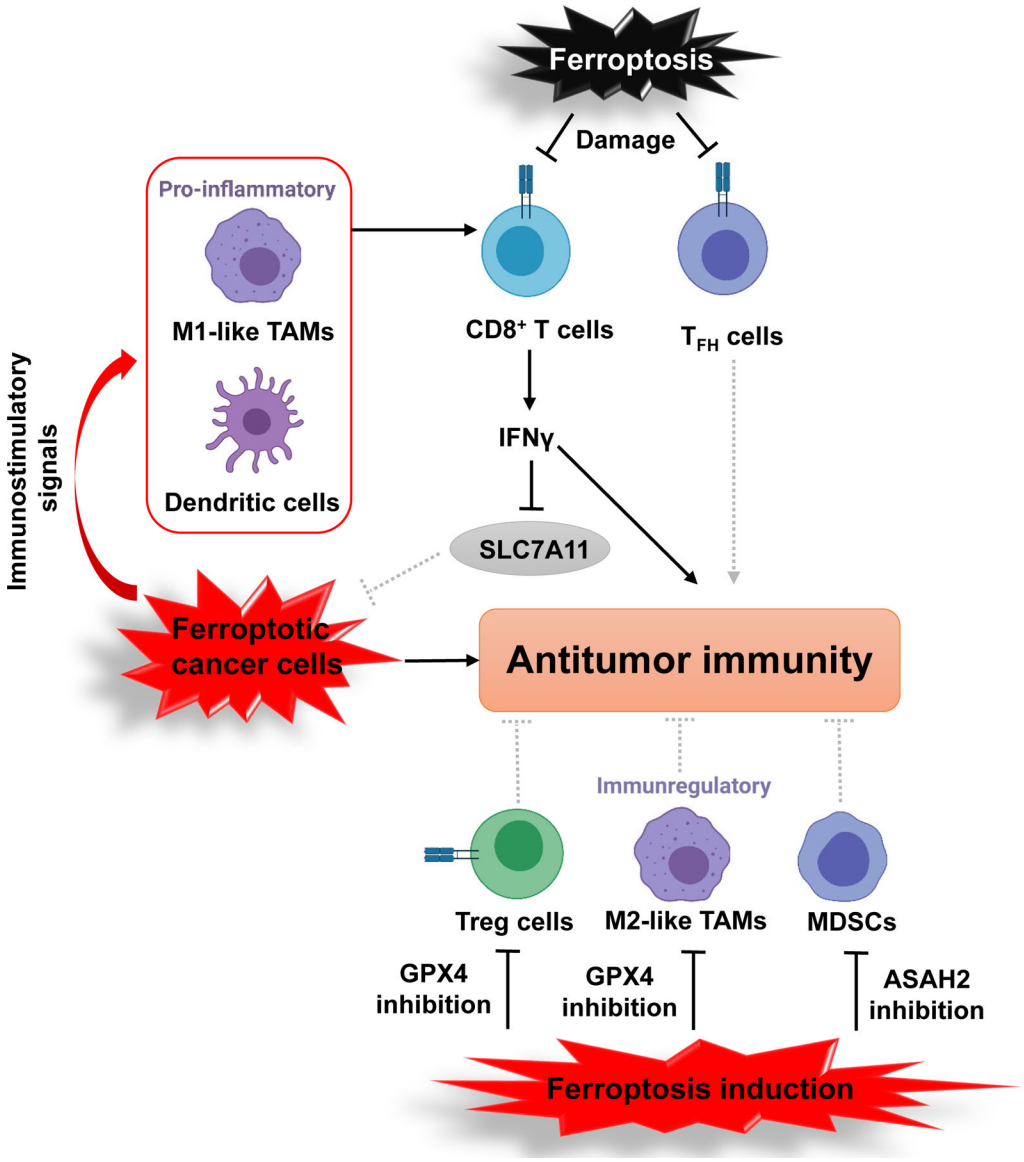

Figure 3. The role of ferroptosis in antitumour immunity.

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 (ASAH2), thereby augmenting antitumour immunity. However, $CD8^+$ T cells and some T helper (T_H) 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. TCR, T cell receptor.

Table 1.
Strategies for targeting ferroptosis in cancer therapy

27-HC, 27-hydroxycholesterol; ALK, anaplastic lymphoma kinase; BSO, buthionine sulphoximine; BH_4, tetrahydrobiopterin; ccRCC, clear cell renal cell carcinoma; CISD2, CDGSH iron-sulfur domain-containing protein 2; DHODH, dihydroorotate dehydrogenase; *EGFR*, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; FINs, ferroptosis inducers; FSP1, ferroptosis suppressor protein-1; GCH1, GTP cyclohydroxylase-1; GPX4, glutathione peroxidase 4; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; ICIs, immune checkpoint inhibitors; *IDH1*, isocitrate dehydrogenase 1; $iPLA_2\beta$, calcium-independent phospholipase $A_2\beta$; KEAP1, kelch-like ECH associated protein 1; MT1G, metallothionein-1G; mTORC1, mTOR complex 1; NE, neuroendocrine; NCSLC, non-small-cell lung cancer; NEDD4, neural precursor cell expressed, developmentally down-regulated 4; NF2, neurofibromin 2; PD1, programmed cell death protein 1; PDK4, pyruvate dehydrogenase kinase 4; PDL1, PD1 ligand 1; 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; *VHL*, Von Hippel-Lindau.

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]
		TNBC	Breast cancer	SLC7A11 inhibitors, GPX4 inhibitors[114]
	Cancer cells with certain genetic mutations	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]
	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]
Re-sensitizing resistant tumours to ferroptosis	Desuppression of PUFA-PL synthesis and peroxidation	$iPLA_2\beta$ overexpression	p53 wild-type cancers	$iPLA_2\beta$ inhibition + TBH[90]
		Adipokine chemerin upregulation	Renal cell carcinoma	Chemerin monoclonal antibody[92]
	Derestriction of the labile iron availability	NFS1 overexpression	Lung cancer	NFS1 inhibition + BSO, erastin or TBH[95]

Strategy	Classification	Feature	Tumour type	Target or agent
		Frataxin overexpression	Multiple cancer types	Frataxin inhibition + erastin[96]
		CISD2 overexpression	Head and neck cancer	Pioglitazone (CISD2 inhibitor) + sulfasalazine (SLC7A11 inhibitor)[97]
		Prominin2 upregulation	Breast cancer	Prominin2 inhibition + SLC7A11 inhibitors or GPX4 inhibitors[98]
	Preventing the upregulation of ferroptosis defence systems	SLC7A11 upregulation	KRAS mutation	SLC7A11 inhibitors[102,103]
		GPX4 upregulation	Cancers chronically exposed to 27-HC	GPX4 inhibition[93]
		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]
	Abrogation of oncogenic activation-mediated ferroptosis resistance	*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]
		PDK4 overexpression	Pancreatic ductal adenocarcinoma	PDK4 inhibitors + SLC7A11 inhibitors[149]
Combining FINs with conventional cancer therapies	Sensitizing conventional cancer therapies	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]
	Overcoming resistance to conventional cancer therapies	Acquired radioresistance, intrinsic radioresistance (*TP53* or *KEAP1* mutation)	NSCLC	RT + SLC7A11 inhibitors or GPX4 inhibitors[25,142,155]
		Acquired chemoresistance (cisplatin or docetaxel)	Head and neck cancer, ovarian cancer	SLC7A11 inhibitors + cisplatin or docetaxel[157,158]
		TYRO3-mediated anti-PD1 or PDL1 therapy resistance	Breast cancer	TYRO3 inhibitors + anti-PD1[159]
		MT1G-mediated sorafenib resistance	Hepatocellular carcinoma	MT1G inhibitors + sorafenib[160]