nature cell biology

Review article

https://doi.org/10.1038/s41556-024-01425-8

Exploiting ferroptosis vulnerabilities in cancer

Received: 9 February 2024

Accepted: 17 April 2024

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Ferroptosis is a distinct lipid peroxidation-dependent form of necrotic cell death. This process has been increasingly contemplated as a new target for cancer therapy because of an intrinsic or acquired ferroptosis vulnerability in difficult-to-treat cancers and tumour microenvironments. Here we review recent advances in our understanding of the molecular mechanisms that underlie ferroptosis and highlight available tools for the modulation of ferroptosis sensitivity in cancer cells and communication with immune cells within the tumour microenvironment. We further discuss how these new insights into ferroptosis-activating pathways can become new armouries in the fight against cancer.

In 2012, a new type of cell death modality was described that was hallmarked by iron-dependent lipid peroxidation and became commonly known as ferroptosis[1]. Ferroptosis is distinctly different from other forms of cell death, such as apoptosis, necroptosis and pyroptosis, as it does not involve any of the well-known essential cell death executors such as cleaved caspases, mixed lineage kinase domain-like pseudokinase (MLKL) and cleaved gasdermin D[2]. Instead, aberrant cellular redox control and overwhelming peroxidation of phospholipids also occurs, which might be triggered in an iron-dependent or even iron-independent manner[1]. Moreover, it is characterized by morphological abnormalities of mitochondria such as rupture of the outer membrane and shrinkage of cristae[1]. Given the rapidly growing interest in ferroptosis, we provide here a brief overview of the underlying molecular mechanisms of ferroptosis and a critical evaluation of its inducers. We also discuss the susceptibility of cancer cells to ferroptosis and the interaction between cancer and immune cells. Finally, we present future considerations that should help guide the design of ferroptosis-based cancer therapies.

Cellular ferroptosis defence systems

The system x_c^-–GSH–GPX4 axis

In the search for new anticancer drugs in the early 2000s, Stockwell and colleagues identified erastin and (1*S*,3*R*)-RSL3 (RSL3) as selective cell-death inducers in oncogenic rat sarcoma virus mutant cells expressing RAS(G12V)[3,4]. Both of these small-molecule compounds triggered a new form of cell death that could be rescued by iron chelators, vitamin E and/or glutathione (GSH) supplementation[4]. As iron is important for this form of cell death, it was named ferroptosis[1] (Fig. 1). Independent studies by our laboratory at the same time provided the first evidence that genetic deletion of *Gpx4* (which encodes glutathione peroxidase 4) in fibroblasts and cortical neurons in vivo triggered an as-yet unrecognized non-apoptotic form of cell death[5]. This process was characterized by lipid peroxidation and was prevented by vitamin E, lipoxygenase inhibitors and suppression of apoptosis-inducing factor mitochondria associated 1 (AIFM1) expression[5]. Although the role of GPX4 in the prevention of phospholipid peroxidation is at the core of ferroptosis, the potential role of AIFM1 in ferroptosis has been refuted[6], and the involvement of lipoxygenase in ferroptosis remains controversial[7]. RSL3 is the first described GPX4 inhibitor and binds covalently to selenocysteine (Sec), which is the active site of GPX4. Sec requires special machinery for its synthesis and co-translational incorporation at the UGA codon (Box 1), and so RSL3 irreversibly blocks its phospholipid and lipid hydroperoxidase activity to suppress ferroptosis[8,9]. GSH is the preferred reducing substrate of GPX4 (ref. 10). Therefore, depletion of intracellular GSH either by inhibiting γ-glutamylcysteine-ligase (γ-GCL)[5] through L-buthionine sulfoximine (BSO) or genetic deletion of γ-GCL ultimately leads to inactivation of GPX4 and ferroptosis in a cell-type and context-dependent manner[11,12]. Alternatively, the availability of cystine, the dimerized oxidized form of cysteine and the building block of GSH, can be limited to induce ferroptosis. Erastin and sulfasalazine (SSZ) can inhibit the uptake of cystine through the cystine–glutamate antiporter designated system x_c^- (a heterodimer consisting of SLC7A11 (xCT) light chain and SLC3A2 (4F2) heavy chain), which ultimately leads to cysteine starvation, depletion of GSH, GPX4 inactivation and ferroptosis[13].

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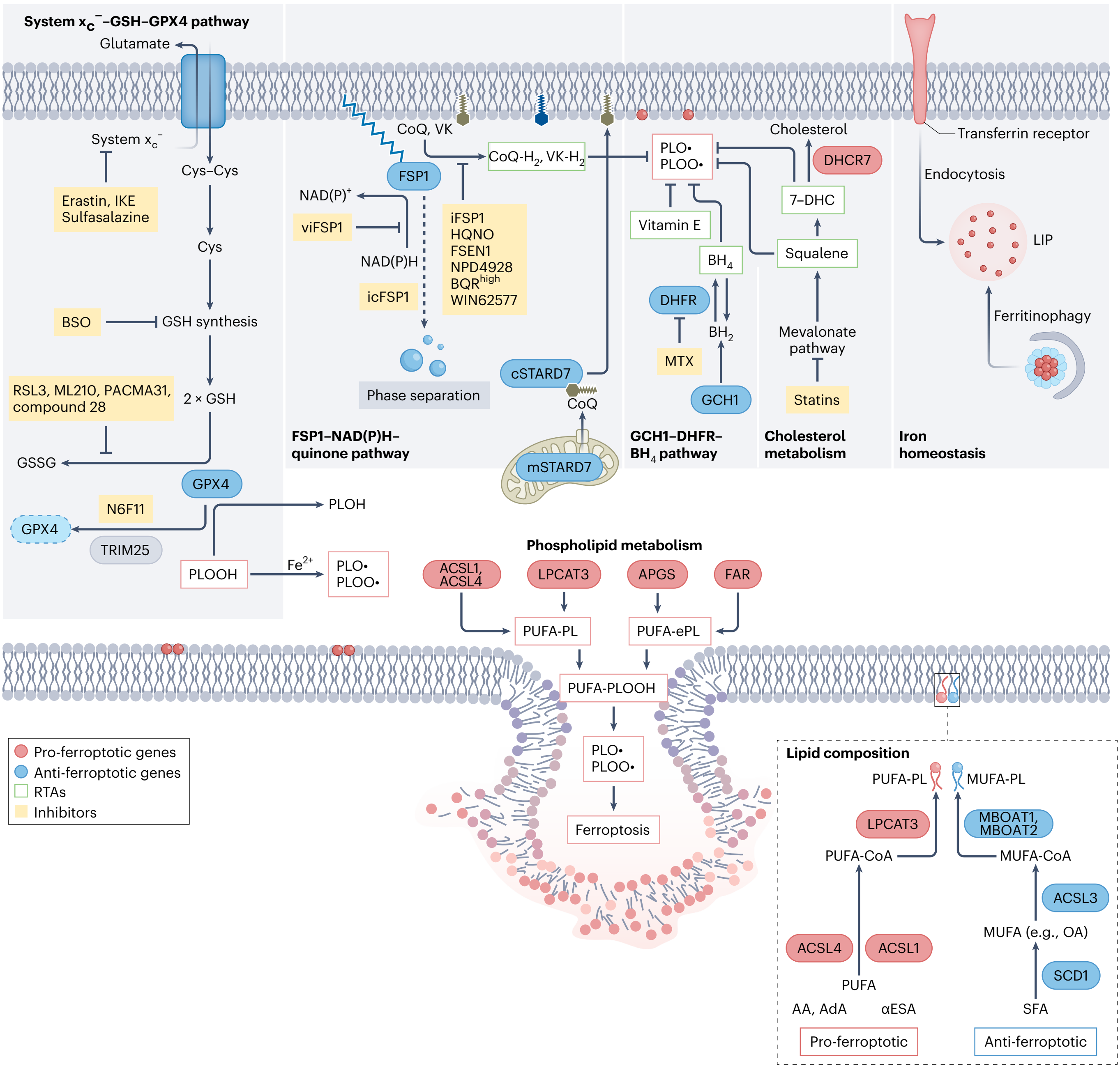


Fig. 1 | Overview of the main pathways that regulate ferroptosis. Ferroptosis is ultimately triggered by uncontrolled phospholipid peroxidation, which leads to disruption of plasma membrane integrity. The system x_c^-–GSH–GPX4 pathway prevents phospholipid peroxidation by directly reducing phospholipid hydroperoxides to the corresponding alcohols, which is achieved by GPX4. The NAD(P)H–FSP1–quinone pathway and the GCH1–DHFR–BH_4 pathway (re)generate exogenous and endogenous RTAs. Intracellular, free redox-active labile iron pool (LIP) can result, for instance, from transferrin receptor-mediated endocytosis of transferrin-bound iron and subsequent reduction to ferrous iron or by ferritin degradation through ferritinophagy. The balance of PUFAs and MUFAs esterified in phospholipids or PUFA-ePLs determines the susceptibility of a cell to ferroptosis. AA, arachidonic acid; AdA, adrenic acid; BQR, brequinar; c/mSTRAD7, cleaved/mitochondrial STRAD7; DHCR7, 7-dehydrocholesterol reductase; αESA, α-eleostearic acid; FAR, fatty acyl-CoA reductase 1; GSSG, glutathione disulfide; MTX, methotrexate; OA, oleic acid; PLO(O)•, phospholipid peroxyl radical.

Moreover, uptake of cystine by system x_c^- can protect cells against ferroptosis in a GPX4-independent manner by increasing the amount of hydropersulfides that act as one-electron reductants to quench free radicals in lipid bilayers[14,15]. Cysteine can also be synthesized from serine and methionine through the cell-type-specific transsulfuration (TSS) pathway[16]. Therefore, sufficient cellular cysteine levels can rescue ferroptosis[17,18]. Together, these studies provide conclusive evidence of the pivotal role that the system x_c^-–GSH–GPX4 pathway has as the primary system that protects cells against ferroptosis (Fig. 1).

The NAD(P)H–FSP1–hydroquinone pathway

In addition to the canonical system x_c^-–GSH–GPX4 axis, two research groups independently identified ferroptosis suppressor protein-1 (FSP1; encoded by *AIFM2*) as an endogenous ferroptosis suppressor in many cancer cells[6,19]. Detailed mechanistic studies uncovered that the anti-ferroptotic function of FSP1 relies on its activity to reduce ubiquinone (also known as coenzyme Q (CoQ)) or vitamin K (VK) to their hydroquinone forms, ubiquinol ($CoQ\text{-}H_2$) or $VK\text{-}H_2$, respectively, which prevent uncontrolled lipid peroxidation by trapping radicals

BOX 1

Selenium metabolism and ferroptosis

Sec is essential for the full activity of GPX4 to enable efficient reduction and scavenging of phospholipid and cholesterol hydroperoxides in lipid membranes to the corresponding alcohols[140]. This in turn is crucial to prevent an uncontrolled lipid peroxidation chain reaction and membrane rupture. Although Sec is essential for both the full activity of GPX4 and protection against peroxide-induced irreversible overoxidation, its utilization in selenoproteins comes at a high price. First, loading of Sec-specific transfer RNA requires the synthesis of Sec on its cognate tRNA TRSP (also known as TRU-TCA1-1) by converting serine-loaded tRNA into Sec-tRNA, which requires a specific cellular machinery[141]. Moreover, decoding of Sec at the UGA opal termination codon necessitates the concerted action of several proteins that recognize a distinct stem–loop-like structure in the 3′ untranslated region of selenoproteins known as the SECIS element, as well as interaction with the stalled ribosome at the UGA codon[141,142]. Therefore, impaired expression of proteins involved in Sec-tRNA synthesis or insufficient selenium availability may increase ferroptosis sensitivity mainly due to GPX4 deficiency[142,143]. In physiology, selenium is normally absorbed in its organic form such as selenomethionine (SeMet) or selenoproteins. Selenoprotein P (SELENOP) is synthesized in the liver, which contains up to ten Sec residues and is then distributed throughout body[144]. The organic pathway depends on SELENOP uptake into tissues, which is mediated by low-density lipoprotein receptor-related protein 2 (LRP2; also known as megalin) and low-density lipoprotein receptor-related protein 8 (LRP8; also known as APOER2)[141,142]. SELENOP is then degraded in the lysosome and Sec is extracted through the endosome–lysosome pathway and cleaved by selenocysteine β-lyase (SCLY) to provide selenium to selenophosphate synthetase 2 (SEPHS2) in the form of HSe to produce selenoproteins including GPX4 (refs. 102,142,145). In addition, an inorganic pathway allows cells to import HSe and/or $SeO_{3/4}^{2-}$ through currently unknown ion channels. However, it remains unclear whether this pathway is relevant for mammals, as inorganic selenium levels are very low in nature[102]. Nonetheless, the amount of extracellular cysteine supported by system x_c^- uptake of cystine, intracellular cystine reduction and secretion through neutral amino acid transporters serves as the reducing power to convert $SeO_{3/4}^{2-}$ to HSe[102,146]. In addition to this, high dose of selenide supplementation can rescue ferroptosis in a GPX4-indendent manner[146]. Mechanistically, sulfide quinone oxidoreductase (SQOR) reduces ubiquinone by oxidizing selenide instead of sulfide in mitochondria. This finding highlights the importance of selenium metabolism as a crucial factor in the regulation of ferroptosis.

in lipid bilayers[6,19,20]. In addition, the anti-ferroptotic function of FSP1 requires the ability to bind membranes through amino-terminal myristoylation[6,19], which is accomplished by *N*-myristoyl transferases (NMTs). CoQ is synthesized in mitochondria from where it then translocates to the cytosol or plasma membrane, along with StAR-related lipid transfer domain protein (STARD7)[21] (Fig. 1). The dependence of cells on the NAD(P)H–FSP1–hydroquinone axis is based on the cell type and cell context and remains to be fully elucidated, particularly in the in vivo setting. This cell dependence is also true for the other ferroptosis surveillance systems, as discussed below. Nonetheless, leukaemic cancer cells and lymphoma cells are sensitive to FSP1 inhibition alone to some extent, and lung cancer cells show comparably high expression. Therefore, whether these cancers should be the first choice for the development of FSP1-based therapies needs to be carefully investigated.

The GCH1–BH_4–DHFR pathway

In addition to the GPX4 and FSP1 pathways, a CRISPR activation screen identified GTP cyclohydrolase 1 (GCH1) as another ferroptosis-suppressing pathway[22]. Mechanistically, GCH1 generates tetrahydrobiopterin (BH_4), which can act as a radical-trapping antioxidant (RTA) and can be recycled by dihydrofolate reductase (DHFR)[23]. The expression of GCH1 and the availability of BH_4 are only abundant in leukaemias and lymphomas. Therefore, interfering with the GCH1–BH_4 pathway could be a potential therapeutic route to successfully treat leukaemia (Fig. 1).

Phospholipid, lipid and cholesterol metabolism

Sensitivity to ferroptosis is highly dependent on the balance of polyunsaturated fatty acids (PUFAs) and monosaturated fatty acids (MUFAs) that are esterified in cell membranes. The incorporation of free PUFAs into cellular membranes is regulated by the sequential action of acyl-CoA synthetase long-chain family member 1 (ACSL1) and ACSL4 and lysophosphatidylcholine acyltransferase 3 (LPCAT3), as well as membrane-bound *O*-acyl-transferase family member 7 (MBOAT7)[24–26]. Moreover, phosphorylation of ACSL4(T328) by protein kinase C (PKCβII) enhances ACSL4 dimerization and activity[27]. Furthermore, the biosynthesis of polyunsaturated ether phospholipids (PUFA-ePLs) by peroxisomal enzymes, such as alkylglycerone phosphate synthase (AGPS) and transmembrane protein 164 (TMEM164)[28,29], are involved in lipid peroxidation in a cell-type-specific manner. High expression and activation of this pathway could therefore be considered a ‘pro-ferroptotic’ state[25,26,28]. By contrast, the abundance of saturated fatty acids (SFAs) or MUFAs in the lipid membrane leads to an ‘anti-ferroptotic’ state of cells[30,31]. Stearoyl-CoA desaturase (SCD1) catalyses the formation of MUFAs from SFAs, and ACSL3 activates MUFA-CoAs[30,32], and their incorporation into membranes is achieved by membrane bound *O*-acyltransferase domain containing 1 (MBOAT1) and MBOAT2 (MBOAT1/2)[33]. Therefore, high expression of the components in the SCD1–ACSL3–MBOAT1/2 pathway may cause resistance to ferroptosis[34].

In addition to phospholipid metabolism, the mevalonate pathway is important for regulating ferroptosis. Blockade of mevalonate metabolism leads to inhibition of CoQ synthesis and Sec-tRNA synthesis (tRNA[Ser]Sec is stabilized by isopentenylation, an intermediate of the mevalonate pathway)[35,36], and therefore directly affects FSP1 activity and GPX4 expression, respectively. Furthermore, modulating the availability of lipophilic, endogenously synthesized RTAs, squalene and 7-dehydrocholesterol (7-DHC) as intermediates of the mevalonate pathway regulates ferroptosis susceptibility[36–41]. In summary, lipid metabolism and associated enzymes are crucial for the regulation of ferroptosis susceptibility.

Iron metabolism

Iron is indispensable for several biological processes, for example for Fe–S cluster biogenesis, haem biosynthesis and the activity of certain enzymes[42]. However, an excess of iron can be deleterious, as small amounts of redox-active iron are contained in the so-called cellular labile iron pool (LIP)[42]. If not properly sequestered in the LIP, redox-active iron can contribute to the formation of highly reactive hydroxyl radicals through Fenton-type chemistry[42], which in turn can initiate phospholipid and lipid peroxidation. The main source of cellular iron is its uptake by transferrin receptor (TFRC)-mediated endocytosis, which is followed by storage in ferritin (FTH and FTL), the main cellular iron storage proteins[42]. Transferrin is a pH-sensitive but reversible ferric iron (Fe^{3+})-binding protein, whereas ferritin is a huge cargo protein that can store thousands of ferric irons[42]. Depending on cellular iron demand, ferritin is selectively degraded (that is, ferritinophagy), which is mediated by the cargo-protein nuclear receptor coactivator 4 (NCOA4)[43]. In addition to these pathways, certain cancer cells and cancer stem cells amplify the expression of CD44, which facilitates the uptake of hyaluronic-acid-bound iron by endocytosis[44]. Notably, all the iron utilization processes in cells require acidification in lysosomes and endosomes.

Ferroptosis inducers

Since the term ferroptosis was coined, the list of ferroptosis-inducing compounds and tools that target pivotal nodes to effectively kill cancer cells has steadily increased (Table 1). However, many mistakes and common misconceptions have also arisen. For example, pharmacological inhibitors with poorly defined mechanism-of-actions or poor pharmacokinetic properties are often used for ferroptosis research. Table 2 provides an overview and clarification of the key resources available in the field.

Nonetheless, there are two main ways to efficiently sensitize cancer cells or trigger ferroptosis-mediated cell death: (1) pharmacological inhibitors or modulators for ferroptosis-related enzymes; and (2) direct induction of the process of lipid peroxidation. To effectively deliver these ferroptosis inducers to tumours, several systems have been developed: (1) chemical modifications to the compounds themselves to improve solubility and metabolic stability in vivo; (2) protein degradation by E3 ligases or autophagy; (3) formulations using liposomes or nanoparticles; and (4) antibody–drug conjugates (ADCs) to increase specific targeting of tumours to enhance efficacy and minimize side effects (Fig. 1). Below, we provide an overview of some of these approaches and discuss their potential for in vivo application.

Inhibitors of the system x_c^-–GSH–GPX4 pathway

Erastin, the most widely used system x_c^- inhibitor in the laboratory, is not suitable for in vivo use. Therefore, efforts in medicinal chemistry have been made to improve its solubility and metabolic stability, which have led, for example, to the development of piperazine erastin and imidazole ketone erastin (IKE)[45]. IKE showed improved metabolic stability and impaired tumour growth in a mouse model of diffuse large B cell lymphoma. In addition, the clinically used sulfasalazine (SSZ) has long been known to be a system x_c^- inhibitor[46]. However, although SSZ inhibits system x_c^- in cell culture at high micromolar concentrations[46], the maximum concentration of SSZ reached in vivo is not sufficient to kill a variety of cancer cells[47,48]. This limits its therapeutic applications, for example, in cancer treatment. For instance, a clinical trial in patients with glioblastoma had to be discontinued because there was no response in patients with malignant glioma and two patients died prematurely[49]. Sorafenib is an anticancer multikinase inhibitor approved by the US Food and Drug Administration (FDA) that purportedly induces cell death through system x_c^- inhibition[13,50]. However, a recent study[47] demonstrated that sorafenib neither inhibits system x_c^- nor induces ferroptosis, which suggests that sorafenib is not a bona fide inducer of ferroptosis. Cyst(e)inase is an engineered human enzyme for the degradation of extracellular cysteine and cystine[51]. Cyst(e)inase reduced tumour growth in xenograft models[51–55] and prolonged survival of mice with chronic lymphocytic leukaemia[52]. In addition, interferon-γ (IFNγ), the cytokine secreted by CD8$^+$ T cells, impairs system x_c^- activity by downregulating its expression in a JAK–STAT-dependent manner, which in turn impairs tumour growth[53,56]. Thus, the combination of immune checkpoint therapies, such as a programmed death-ligand 1 (PD-L1) antibody, together with cyst(e)inase or other ferroptosis inducers may represent a new paradigm for cancer therapy[53].

BSO is a long-known, specific inhibitor of γ-GCS that deprives GSH and induces ferroptosis[57]. Although BSO is an orally available drug, BSO treatment alone fails to suppress tumour growth and rapidly leads to drug resistance[11,58]. The first-generation GPX4 inhibitor RSL3 works well in cell culture but is unsuitable for in vivo use owing to its poor metabolic stability and other pharmacokinetic limitations. Moreover, RSL3 targets almost all selenoproteins (Box 1) because of its strongly electrophilic warhead[59]. Therefore, data obtained in cell culture using RSL3 requires careful interpretation. Although second-generation GPX4 inhibitors, including ML210 and its derivatives such as the orally available JKE-1674, have been developed, a higher dose of these is required for ferroptosis induction[60]. Several other GPX4 inhibitors, such as compound C18, compound 28 and PACMA31, have been developed as in vivo GPX4-binding drugs for the treatment of tumours with higher potency[61–64]. Notably, when PACMA31 is combined with the FDA-approved multikinase inhibitor regorafenib, ferroptotic cell death is synergistically induced and tumour growth is impaired[63]. As GPX4 inhibitors have inherent off-target effects on other selenoproteins, targeting allosteric sites of other cysteine residues within the GPX4 protein can be an alternative approach. For example, LOC1886 is an allosteric GPX4 inhibitor[65]. However, as concentrations in the 100 µM range are required to inhibit GPX4, this compound is probably unsuitable for in vivo use at this stage. FIN56 and DMOCPTL induce autophagic and proteasomal degradation of GPX4, respectively, but with unknown mechanisms[36,66,67]. However, FIN56 is metabolically unstable and shows off-target effects on squalene syntheses, whereas DMOCPTL also induces apoptosis. Thus, both compounds may not be suitable for therapeutic application. Altretamine, a FDA-approved anticancer drug, inhibits GPX4 activity[68], although its mechanism-of-action remains obscure. Other than these inhibitors, some compounds have been shown to inhibit GPX4 directly or indirectly[8,69], but their actual in vivo potential requires further study.

FSP1–NAD(P)H–CoQ pathway inhibitors

As GPX4 inhibition is often insufficient to trigger cell death in certain cancer cells, simultaneous inhibition of FSP1 and GPX4 has been considered a more effective strategy for cancer therapy[6,19]. One of the first described FSP1 inhibitors iFSP1 indeed shows synergistic effects with GPX4 inhibition in a wide range of human cancer cells[6], and it works by targeting the quinone-binding pocket[70]. However, it is specific for the human orthologue, which makes animal studies difficult[70,71]. The FSP1 inhibitors NPD4928, FSEN1 and WIN62577 exhibit similar half-maximum effective concentration (EC_{50}) values to iFSP1, but their respective mechanism-of-action remains unclear[9,72,73]. HQNO and other ubiquinone derivatives, such as quinolinyl pyrimidine, are reported to inhibit FSP1 to some extent[74,75]. Similarly, brequinar and some mitochondrial ubiquinol-reducing enzyme inhibitors that target dihydroorotate dehydrogenase (DHODH) also inhibit FSP1 at higher concentrations[71]. The first cross-species FSP1 inhibitor, versatile inhibitor of FSP1 (viFSP1), inhibits FSP1 presumably by targeting the NAD(P)H-binding pocket[70]. The first human-specific FSP1 inhibitor applicable in vivo, inducer of condensates of FSP1 (icFSP1), has a distinct mechanism of action[12]. In contrast to other FSP1 inhibitors, icFSP1 does not directly inhibit FSP1 activity but inactivates FSP1 by forcing it to move away from the plasma membrane and induces phase separation of FSP1 (ref. 12). In synergy with genetic deletion of *GPX4*, icFSP1 limits tumour growth in various tumour models[12]. This study provides the basis for the development of efficient anticancer therapies by targeting FSP1-dependent phase separation.

Endoperoxides

The natural product artemisinin and its derivatives, the sesquiterpene endoperoxides (R_1-O-O-R_2), which are the gold standard for malaria treatment, lead to the formation of free radicals that are capable of alkylating various biological targets and initiating lipid peroxidation in the presence of iron(II) or haem[72,76,77]. Other endoperoxide-containing compounds, $FINO_2$ and its derivates, can also induce ferroptosis by increasing lipid peroxidation along with iron oxidation[78,79].

Table 1 | Commonly used ferroptosis-inducing agents

Type	Name	Mechanism of action	Potential and limitations	In vivo use (Y, N or ?)[a]	Refs.
Small molecules	RSL3	Covalent binding to Sec of GPX4	Also targets other selenoproteins; only for cell culture; not stable in vivo	N	4,8
	ML162	Covalent binding to Sec of GPX4	Also targets other selenoproteins; only for cell culture	N	8
	ML210 and JKE-1674	Covalent binding to Sec of GPX4	JKE-1674 is orally available and shows higher stability than ML210	?	60,147
	Compound C18	Covalent binding to Sec of GPX4	Low EC_{50} (10 nM) in TNBCs	Y	62
	Compound 28	Covalent binding to Sec of GPX4	GPX4 engagement can be observed in tumour samples	Y	61
	N6F11	TRIM25-dependent GPX4 degradation	Tumour-specific GPX4 degradation in a TRIM25-dependent manner	Y	90
	PACMA31	Covalent binding to Sec of GPX4	Synergistic effects with regorafenib	Y	63,64
	LOC1886	Covalent binding to Sec of GPX4	Very high EC_{50} values	N	65
	Erastin	System x_c^- inhibition	Unstable in vivo; has off-target effects at high concentrations	N	1,13
	IKE	System x_c^- inhibition	Higher stability in vivo; nanoparticle formulation may be required	Y	45
	BSO	γ-GCS inhibition and GSH production	Orally available; GSH depletion alone is not sufficient to induce ferroptosis in general	Y	11,57,58
	iFSP1	FSP1 inhibition; binding to the quinone binding pocket	Poor solubility and stability in vivo; F360-dependent (human and chicken); off-target effects at high concentrations	N	6,12,70
	viFSP1	FSP1 inhibition; binding to the NAD(P)H binding pocket	A multispecies FSP1 inhibitor	N	70
	icFSP1	Phase separation of FSP1	Inhibits human FSP1 in vivo but not in cell-free systems	Y	12
	FSENs	FSP1 inhibition	Human-specific only; FSEN1 might be useful in vivo	Y?	72
	NPD4928 and WIN62577	FSP1 inhibition	Inhibits human FSP1 in cells and in vitro	?	9,73
	Brequinar	DHODH inhibition (>10 nM) and FSP1 inhibition (>10 μM)	Concentrations need to be carefully controlled	Yes	71,148
	Statins	HMGCR inhibition	Affects the mevalonate pathway, including GPX4, CoQ_{10}, 7-DHC and squalene synthesis	Yes	35,36
	FIN56	GPX4 degradation and activation of squalene synthesis	Multitarget ferroptosis inducer	?	36
	Methotrexate	DHFR inhibition	Specific for lymphoid cells	Y	23
	FINO2	Radical initiator (endoperoxide)	Possible to target specific organelles using its derivatives	?	78,79
	Artemisin derivatives	Radical initiator (endoperoxide)	Standard treatment for malaria; synergistic effects with GPX4 and FSP1 inhibition	Y	72,76,77
Protein	IFNγ	SLC7A11 downregulation	–	Y	53
	Cyst(e)inase	Degradation of cyst(e)ine	–	Y	51
PROTACs	dGPX4@401-TK-12	GPX4 degradation using ML162	Nanoparticles are required for specific delivery to tumour site	Y	91
Antibody	PD-L1 and CTLA-4 antibody	Stimulates IFNγ-mediated ferroptosis through SLC7A11 downregulation	Effective cancer therapy	Y	53,131
ADC	Trastuzumab with RSL3	HER2 antibody conjugated with RSL3	The first ADC combining a GPX4 inhibitor with a HER2 antibody; it may increase tumour specificity	U	88
Off-target-mediated ferroptosis inducers	Withaferin A	GPX4 degradation; iron increase through HO-1 upregulation; apoptosis induction	Multiple targets and its precise mechanism-of-action remain elusive	Y	80
	Sulfasalazine (SSZ)	Anti-inflammatory effects; inhibition of system x_c^-	In vivo concentrations may not reach concentrations sufficient to inhibit system x_c^- for ferroptosis (C_{max} is less than 100 μM in plasma)	N	46,47,149
	Eprenetapopt	Mutant p53 inhibitor; GSH depletion; NFS1 inhibition	In phase 3 clinical trials	Y	81–83
	Altretamine	DNA alkylation; GPX4 inhibition	FDA-approved drug, but in vivo concentrations may not be sufficient to inhibit GPX4	Y	68
	Combination of JQ1 (OTX015) and bortezomib	GPX4 downregulation through BRD4 and proteasome inhibition	GPX4 downregulation; works in vivo; mechanism remains unclear	Y	99
	Cisplatin	DNA alkylation; GSH depletion	Cisplatin forms complexes with GSH	Y	133
Not related to ferroptosis	Sorafenib	Multikinase inhibitor	Neither inhibition of system x_c^- nor ferroptosis induction in cancer cells	N	47
Ferroptosis inhibitor	Liproxstatin-1	RTA	Inhibits tumour growth in initial growth phase by suppressing ferroptosis in neutrophils; its mechanism-of-action remains unclear at later phases of tumour development	?	121,150

[a] Yes (Y), no or unclear (N), or uncertain (?) for in vivo application. C_{max}, maximum plasma concentration. EC_{50}, half-maximal effective concentration.

Table 2 | Important issues associated with key ferroptosis resources in the field

Careful considerations	Common misuses	Problem	Solution	Refs.
On-target or off-target effects of pharmacological ferroptosis inducers	High dose of GPX4 inhibitors and in vivo application	GPX4 inhibitors (for example, RSL3 and ML210) can also inhibit or bind other selenoproteins and cysteine; RSL3 is not suitable for in vivo use	Less than 5 μM for RSL3 should be used in vitro; RSL3-induced cell death needs to be rescuable by ferroptosis inhibitors; RSL3 should be avoided for in vivo use; alternatives should be considered as outlined in Table 1	59
	High dose of brequinar	This bona fide DHODH inhibitor also inhibits FSP1 at higher concentrations (>60 μM)	Low dose of brequinar (<1 μM) or more specific DHODH inhibitors (for example, BAY-2402234) should be used	71
	Sorafenib as system x_c^- inhibitor	Sorafenib cannot inhibit system x_c^- in most of cell lines	Other inhibitors (for example, erastin or SSZ) should be used for system x_c^- inhibition; note that the inhibitory range of SSZ is small	47
Off-target effects of pharmacological inhibitors	Inhibitor off-target activity due to potential RTAs	Several inhibitors, such as LOX inhibitors (for example, PD146176 and baicalein) and MEK inhibitors (such as U0126), can protect cells against ferroptosis regardless of its targeting enzymes	Genetic perturbations and several inhibitors having different moieties and displaying different mechanism-of-action but targeting the same enzyme should be examined	38
Species-dependent effects	iFSP1 for rodent models	Several FSP1 inhibitors (for example, iFSP1, FSEN1 and icFSP1) do not inhibit mouse FSP1; iFSP1 shows off-target activity higher than 10 μM	viFSP1 should be used as a rodent model FSP1 inhibitor but not for in vivo models; iFSP1 should be used at less than 10 μM	6,12,70
Functional assays	GPX4 activity assay using whole-cell lysate	Whole-cell lysates cannot distinguish between GPX4-specific activity or other enzymatic activities	Pull-down enzyme or recombinant enzyme containing Sec should be used for GPX4 activity assays	9

Other ferroptotic modulators

Methotrexate, an approved drug for chemotherapy and immune suppression and is an inhibitor of DHFR, acts synergistically with GPX4 inhibition to induce ferroptosis specifically in lymphoid cells[23]. Withaferin A is a dual-function ferroptotic inducer; it can inhibit both GPX4 and kelch like ECH associated protein 1 (KEAP1)[80]. Inhibition of the rate-limiting enzyme of the mevalonate pathway (HMG-CoA reductase) by statins indirectly affects various ferroptosis regulators[35,36]. This consequently renders cells susceptible to ferroptosis by limiting the translation of GPX4, the synthesis of CoQ and that of other endogenously synthesized RTAs, such as 7-DHC and squalene[37,39–41]. Eprenetapopt, which is in phase 3 clinical trials, can deplete GSH to induce ferroptosis[81,82] and inhibit cysteine desulfurase (NFS1)[83]. It can also reactivate mutant p53, which has been shown to transcriptionally repress *SLC7A11* expression[84,85]. Therefore, eprenetapopt may have multiple functions that target p53 and NFS1 to induce ferroptosis.

In summary, since the discovery of ferroptosis inducers targeting GPX4 and system x_c^- almost 20 years ago[3,4], substantial effort has been devoted to developing ferroptosis modulators as anticancer drugs. Some of these have been tested in in vivo models and show promising results. However, none of them has gone into clinical trials. Therefore, additional modifications on existing compounds or alternative approaches should be considered.

ADCs, nanoparticles and PROTACs

As discussed above, numerous compounds have been used to modulate ferroptosis. To specifically target ferroptosis-inducing compounds to the site of the tumour and to improve pharmacokinetics, newly developed approaches are being exploited. Among them, the use of ADCs, which combine specific monoclonal antibodies with cytotoxic agents, is particularly promising in terms of increased tumour specificity[86]. Loss of *Gpx4* can lead to embryonic lethality, fatal acute renal failure and neurodegeneration[87]; therefore, this strategy could be particularly beneficial for inhibitors that target GPX4 (ref. 87). An ADC that uses trastuzumab and RSL3 as a payload has been reported[88], but its applicability remains unclear. Alternatively, nanoparticles offer a potentially effective means of killing cancer cells while limiting side effects.

Nanoparticles loaded with ferroptosis-inducing compounds can exhibit enhanced metabolic stability and increased cytotoxicity towards tumours in vivo. Cytotoxicity is triggering either through Fenton-type chemistry by delivering iron[89] or directly inducing ferroptosis[45]. Taking advantage of tumour-specific marker proteins would be the ideal strategy to kill tumours by ferroptosis without affecting immune cells or other normal tissues. In this regard, N6F11 serves as a good example as it triggers the degradation of GPX4 in a manner that depends on the RING domain of E3 ubiquitin ligase tripartite motif containing 25 (TRIM25)[90]. TRIM25 is specifically expressed in tumours, so N6F11 does not exhibit any cytotoxicity against immune cells.

A more direct strategy to degrade GPX4 is the proteolysis targeting chimera (PROTAC) method. In PROTAC, the respective inhibitor is conjugated to a ligand of an E3 ligase to recruit the target protein for ubiquitin-mediated degradation. This was exemplified for dGPX4 as a promising GPX4 degrader[91]. Embedded in reactive-oxygen-species-responsive degradable lipid nanoparticles (dGPX4@401-TK-12), GPX4 was shown to be degraded preferentially in tumours, thereby limiting tumour growth[91]. Therefore, combining these delivery systems with ferroptosis inducers could be a powerful approach in cancer therapy, especially for those compounds that target essential ferroptosis-related enzymes that are required for tissue homeostasis and protection.

Ferroptosis susceptibilities in cancer

Since the discovery of ferroptosis, oncogenic cancer cells have been found to be susceptible to this type of cell death in various tumour entities[1]. This in turn has prompted numerous studies to investigate how ferroptosis can be exploited for cancer therapy. Therefore, a better understanding of which cancer cells are primarily susceptible to ferroptosis and which cancer types are resistant is crucial for the efficient use of ferroptosis-inducing compounds in treating specific cancer types (Fig. 2). Below, we discuss the following aspects: (1) therapy-refractory and mesenchymal cancers as promising targets of ferroptosis; (2) effect on ferroptosis sensitivity derived from oncogene addiction; and (3) the tumour microenvironment (TME) restraining ferroptosis vulnerability.

Persister and de-differentiated cancer cells

Resistance to chemotherapy is one of the most challenging clinical problems in cancer. Notably, certain malignant cancer cells acquire an intrinsic vulnerability to ferroptosis during acquisition of a

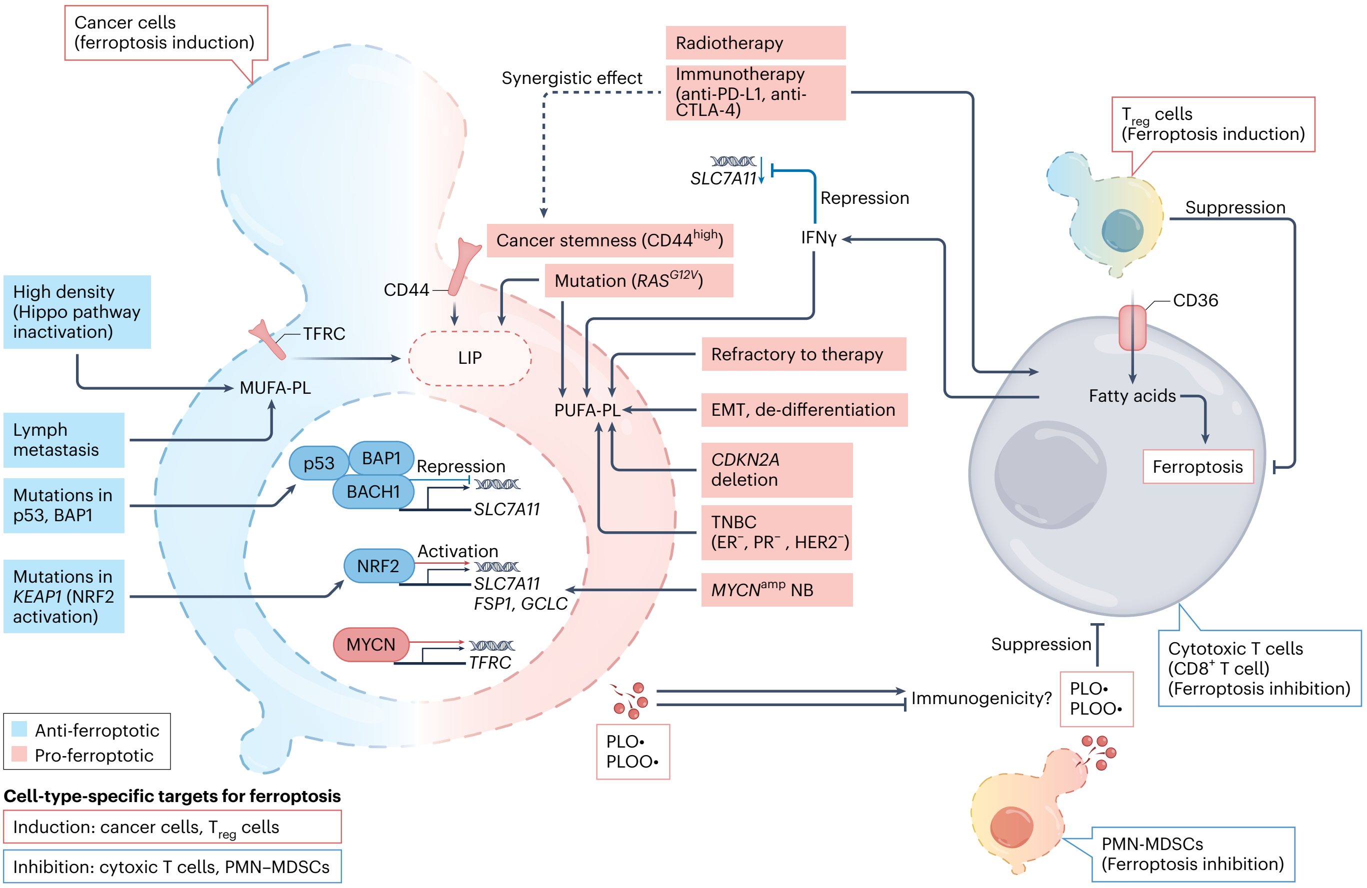


Fig. 2 | Crosstalk between ferroptotic cancer cells and the immune system. Cancer cells exhibit different vulnerabilities to ferroptosis. Genetic mutations and microenvironments modulate their susceptibility to ferroptosis by perturbing iron uptake, phospholipid and lipid composition and expression of either anti-ferroptotic or pro-ferroptotic genes (indicated in blue or red, respectively). The effects of lipid peroxidation originating from ferroptotic cancer cells on tumour immunogenicity remain unclear. Cytotoxic T cells secrete IFNγ and promote tumour ferroptosis through *SLC7A11* downregulation (system x_c^-–GSH–GPX4 pathway) and *ACSL4* upregulation (phospholipid metabolism). Regulatory T (T_{reg}) cells and ferroptotic PMN-MDSCs inhibit cytotoxic T cell function, which leads to the reduction of anti-tumour immunity and impairing tumour growth. MUFA-PL, monounsaturated fatty acid-phospholipid; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CD44^{high}, high expression of CD44; PUFA-PL, polyunsaturated fatty acid-phospholipid; *MYCN*^{amp} NB, MYCN amplified in neuroblastoma.

mesenchymal state or metabolic rewiring. Three initial studies have shown that drug-tolerant and mesenchymal states of cancer cells make them more vulnerable to ferroptosis, particularly when GPX4 is inhibited[35,92,93]. Related to the cancer cell state, cancer stemness, characterized by high CD44 expression, also increases ferroptosis sensitivity through its high iron abundance, which is mediated by endosomal uptake through CD44 itself[44]. In addition, the induction of sublethal apoptosis, which is associated with mitochondrial outer membrane permeabilization (MOMP) and cytochrome *c* release from mitochondrial to activate caspase-3, leads to a metabolic switch and increases ferroptosis susceptibility[94]. These studies suggest that targeting ferroptosis is a promising approach to combat chemotherapy-resistant and mesenchymal tumours.

Indeed, high metastatic capacity, such as the induction of epithelial–mesenchymal transition (EMT), certainly makes cancer cells more vulnerable to ferroptosis. However, the susceptibility of cancer cells to ferroptosis varies according to the metastatic pathway and environmental conditions. Several studies have shown that cancer cells in blood are exposed to increased oxidative stress[31,95,96]. By contrast, the high MUFA content in lymph and lymph nodes confers high resistance to ferroptosis to resident cancer cells[31], as shown earlier[30]. Therefore, treatment of lymphatic metastatic cancer cells will probably require a specific approach to avoid inducing ferroptosis resistance.

Oncogene addiction in ferroptosis sensitivity

Ferroptosis inducers were initially discovered in HRAS-mutated engineered human fibroblasts, and their effectiveness was subsequently confirmed in cells expressing other oncoproteins, including KRAS and NRAS[4,97]. Since then, an increasing number of oncogenes have been reported to affect ferroptosis sensitivity, typically by modulating the expression of key ferroptosis genes. First, the *HRAS*^{G12V} mutant, the most common mutation in *HRAS* and constitutively active form of HRAS, induces transcriptional activation of iron-metabolism-related genes in transformed fibroblasts[4]. Meanwhile, *KRAS*^{G12V} alters lipid profiles in lung cancer[98]. A subset of triple-negative breast cancers (TNBCs), which harbour deletions of the oestrogen receptor (ER) and progesterone receptor (PR) and amplified HER2 (encoded by *ERBB2*), the upstream regulator of the mitogen-activated protein kinase (MAPK) pathway, is sensitive to ferroptosis owing to its high expression of ACSL4, which results in a high PUFA content[25,99]. The gain-of-function mutants of tumour protein 53 (p53), the best-studied tumour-suppressor protein, has been shown to regulate (at least transiently) the expression of *SLC7A11* by directly interacting with BTB domain and CNC homologue 1 (BACH1) through its binding to the transcription start site of *SLC7A11* (refs. 84,85). Furthermore, BRCA1-associated protein 1 (BAP1) suppresses *SLC7A11* expression by binding to its promoter region, whereas cancer-associated mutations of *BAP1* sustain *SLC7A11* expression[100]. The oncogenic transcription factor MYCN affects ferroptosis sensitivity in multiple ways. MYCN directly binds to the *TFRC* promoter, and *MYCN*-amplified neuroblastoma increases iron[101]. In addition, *MYCN*-amplified neuroblastoma contains high cysteine through high expression of *SLC3A2* and activation of the TSS pathway. Furthermore, *MYCN*-amplified neuroblastoma relies on LRP8-mediated selenium uptake for its selenium source[102,103]. Comprehensive genome and lipidome meta-analyses revealed that deletion of cyclin-dependent kinase inhibitor 2 A (CDKN2A) reshapes the cellular lipidome and renders glioblastoma susceptible to ferroptosis[104]. Numerous cancer cells express FSP1, and its high expression correlates strongly with resistance to ferroptosis inducers[6,19,47]. In particular, high FSP1 expression in non-small cell lung cancer[105,106] and pancreatic ductal adenocarcinoma[107] is associated with activation of the KEAP1-nuclear factor erythroid 2-related factor 2 (NFE2L2; also known as NRF2) system[107]. KEAP1 acts as an adaptor protein required for ubiquitin-dependent degradation of NRF2, whereas *KEAP1* mutations abrogate NRF2 ubiquitination. This then causes constitutive activation of NRF2, which leads to high expression of ferroptosis-related genes such as *SLC7A11*, *GCLC* (which encodes glutamate-cysteine ligase catalytic subunit), *GCLM* (which encodes glutamate-cysteine ligase modifier subunit), *AIFM2*, *FTH1* and *FTL*[105,106]. Another potential regulator of *AIFM2* in cancer is bromodomain-containing 4 (BRD4), which is a member of the bromodomain and extra terminal domain protein family (BET). Bromodomain-containing proteins abnormally regulate pro-survival genes in cancer[108]. Accordingly, several studies have shown that the BET inhibitor JQ1 (ref. 108) increases ferroptosis sensitivity[99] and downregulates *AIFM2* expression[109].

Role of cell density and oxygen in ferroptosis sensitivity

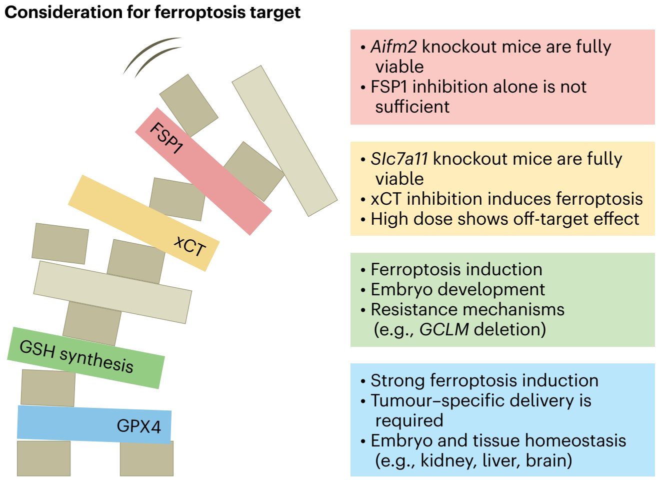
In solid tumours, cancer cells are present at high cell densities and exposed to varying oxygen concentrations. Both factors have a major effect on ferroptosis vulnerability. For example, the transcription factors Yes-associated protein 1 (YAP) and WW-domain-containing transcription regulator 1 (TAZ) translocate to the nucleus at a low cell density and promote the expression of ferroptosis-sensitizing genes such as *TFRC*, *ACSL4* and *ZEB1* (which encodes zinc finger E-box binding homeobox 1)[110,111]. By contrast, when cells are in high density, a pathway that is dependent on cell–cell contacts, comprising NF2, MST1, MST2, LATS1 and LATS2, is activated and phosphorylates YAP and TAZ to induce their translocation out from the nucleus, which leads to a decrease in the expression of ferroptosis-sensitizing proteins. Alternatively, redox-dependent mechanisms may also link cell density to vulnerability to ferroptosis. For instance, Burkitt's lymphoma, a MYC-driven and perhaps the fastest growing tumour in humans, can rapidly undergo oxidative cell death (that is, ferroptosis) when plated under non-permissive conditions such as low cell density, which is due to impaired cysteine uptake[112]. Accordingly, forced expression of either xCT or GPX4 in Burkitt's lymphoma cells is sufficient to drive a cystine–cysteine redox cycle that efficiently protects against ferroptosis by plating cells at low cell density[18,113]. In this context, inhibiting this cystine–cysteine cycle through xCT inhibition can be a therapeutic approach to both affect the TME and reduce GSH levels in the cancer cells per se, provided in vivo active xCT inhibitors are available.

Ferroptosis is driven by oxidative stress; therefore, low oxygen levels, such as hypoxia, is proposed to affect ferroptosis sensitivity in tumours. Regarding oxygen levels, it is questionable whether hypoxia increases susceptibility to ferroptosis. Several studies demonstrated that hypoxic conditions (1–3% oxygen) do not lead to increased ferroptosis sensitivity[13,83,114]. Mechanistically, hypoxic conditions activate hypoxia-inducible factor (HIF-1α) signalling and increase the expression of the glutamate transporter (SLC1A1), leading to increasing cystine uptake by xCT[114]. By contrast, in breast cells or lung cancer cells, ferroptosis inducers, along with depletion of NFS1, the activity of which is essential for the biosynthesis of Fe–S clusters, show a synergistic reduction in cell viability under hypoxic conditions and in tumour growth[54,115]. In clear-cell renal cell carcinomas, activation of HIF-2α is sufficient to enhance the expression of hypoxia-inducible, lipid droplet-associated protein (HILPDA), leading to accumulation of PUFAs in lipid droplets and an increase in susceptibility to ferroptosis[116]. Therefore, the role of hypoxia in ferroptosis susceptibility in an in vivo context remains to be explored.

Ferroptosis and immune cells

There is an intricate crosstalk between cancer cells and immune cells in the TME. Recent work has shed light on how cancer cells and immune cells that undergo ferroptosis affect tumour development and have proposed that activation of ferroptosis in cancer cells could be harnessed for successful immunotherapy against cancer (Fig. 2). Inducing immunogenic cell death might be an optimal approach for cancer therapy because the first hit of cell death by the therapy itself, such as using chemical compounds, and the second hit from immune cells activated by dead cells can eliminate cancer cells from tumours and therefore synergistically result in tumour suppression. One of the first studies that identified a possible link between cancer cells undergoing ferroptosis and tumour immunogenicity suggested that early ferroptotic cells in fibrosarcoma and glioma, which were treated with RSL3, trigger phenotypic maturation of bone-marrow-derived dendritic cells and induce vaccine-like anti-tumour immunity[117]. However, ferroptotic tumour cells present oxidized phospholipids in their plasma membrane that may serve as 'eat me' signals recognized by macrophages, although this concept remains to be fully established[118]. By contrast, another report showed that compared with apoptotic and necroptotic cell death, ferroptosis-derived damage-associated molecular patterns do not elicit an anti-tumour immune response[119]. Moreover, phagocytosis of ferroptotic cancer cells impaired the maturation of dendritic cells[119]. Given these contradictory findings, it remains unclear whether cancer cells undergoing ferroptosis are indeed immunogenic and induce an anti-tumour response, which requires further in-depth investigations.

In addition to specifically triggering ferroptosis in the tumour per se, protecting immune cells against ferroptosis could be a strategy to enhance the immune response to a tumour and support its eradication, as investigated in several studies. Tumour polymorphonuclear neutrophils (PMNs), termed myeloid-derived suppressor cells (PMN-MDSCs), have a suppressive role in T cell proliferation that leads to reduced anti-tumour immunity[120]. Recent work has indicated that tumour-associated PMN-MDSCs are susceptible to ferroptosis and that lipid hydroperoxides derived from these cells undergoing ferroptosis within tumours inhibit T cell proliferation[121]. Therefore, protecting tumour PMN-MDSCs against ferroptosis might increase enhanced anti-tumour immunity by inducing T cell proliferation. In this scenario, inhibiting ferroptosis in the tumour-infiltrating neutrophils from oxidative-stress-induced cell death, including ferroptosis, might be a therapeutic strategy[121,122]. By contrast, there is a controversial finding that tumour-infiltrating neutrophils are resistant to ferroptosis[123]. This is due to the high expression of aconitate decarboxylase 1 (ACOD1), which produces itaconic acid that activates NRF2 by directly modifying KEAP1 (refs. 123,124). Therefore, the advantages of targeting ferroptosis in neutrophils remains unclear. In terms of other immune cells, some studies have shown that CD36-mediated uptake of fatty acids and oxidized low-density lipoproteins increases ferroptosis sensitivity of $CD8^+$ T cells[125,126]. Oxidized low-density lipoproteins induce lipid peroxidation and downstream activation of the apoptosis signal-regulating kinase 1 (ASK1)–p38 pathway[127], thereby impairing $CD8^+$ T cell function such as cytokine production[125,126]. Accordingly, deletion of CD36 or overexpression of GPX4 can protect $CD8^+$ T cells against lipid peroxidation and ferroptosis, thereby enhancing their anti-tumour immune response. Similarly, T regulatory (T_{reg}) cells play a pivotal part in suppressing the anti-tumour immunity of $CD8^+$ T cells through secreting immunosuppressive cytokines such as TGFβ[128]. Therefore, deletion



- *Aifm2* knockout mice are fully viable
- FSP1 inhibition alone is not sufficient

- *Slc7a11* knockout mice are fully viable
- xCT inhibition induces ferroptosis
- High dose shows off-target effect

- Ferroptosis induction
- Embryo development
- Resistance mechanisms (e.g., *GCLM* deletion)

- Strong ferroptosis induction
- Tumour-specific delivery is required
- Embryo and tissue homeostasis (e.g., kidney, liver, brain)

Fig. 3 | Perspectives for targeting ferroptosis in cancer. Potential side effects towards normal tissues and cells when targeting the different ferroptosis nodes in cancer. It can be assumed that targeting only FSP1 or system x_c^- alone has no or only minor side effects. By contrast, targeting GPX4 or GSH biosynthesis, such as GCLC, is likely to be deleterious to multiple organs as symbolized by a toppling over Jenga tower, which therefore requires careful consideration concerning the specificity and therapeutic window.

of *Gpx4* in T_{reg} cells can bolster anti-tumour immune responses and therefore help reduce tumour growth[129].

Finally, several studies have reported that cancer therapies, including chemotherapy, immunotherapy and radiotherapy, can increase the vulnerability of cancer cells to ferroptosis after immune responses through IFNγ secretion[53,106,130,131]. For example, the anticancer drug cisplatin forms complexes with GSH and directly reduces intracellular GSH content in tumours through ATP-dependent transporter efflux[132]. In the TME, IFNγ secreted by $CD8^+$ T cells represses the expression of system x_c^- in fibroblasts and so decreases GSH in the environment. This in turn leads to an increase in cisplatin sensitivity because cisplatin is not exported as a GSH conjugate in cancers[133]. Similarly, IFNγ secreted from activated effector T cells after immunotherapy and/or radiotherapy reduces the expression of system x_c^- and concomitantly increases the expression of *ACSL4* in tumour cells to enhance ferroptosis. In this scenario, targeting FSP1 together with immunotherapy and radiotherapy could be beneficial for effective tumour control[106,130].

Recent studies have been illuminating the complicated interplay between cancer cells and immune cells in the TME, paving the way for cancer therapy by exploiting ferroptosis in either cancer cells or immune cells. However, there are still many unknowns and controversial data, therefore further research is required.

Conclusions and future considerations

Targeting ferroptosis is emerging as a highly attractive and powerful therapeutic strategy to effectively combat certain cancers, especially those that remain difficult to treat, such as metastatic cancers and cancers resistant to standard therapy. Compared with other cell death modalities, ferroptosis, unlike apoptosis and perhaps necroptosis, is probably not induced under physiological conditions, which enables a broad therapeutic window. Ferroptosis is not specific to a particular cell type, such as pyroptosis in immune or epithelial cells[134]. However, it remains to be fully elucidated whether ferroptotic cell death is an immunogenic type of cell death like necroptosis, which would be highly beneficial for efficient anticancer therapy[117,119]. Finally, certain types of cancer cell states, such as metastatic and therapy-resistant so-called persister cells, have emerged to show high vulnerability to ferroptosis, thereby offering the unique opportunity of treating difficult types of cancer[35,92].

BOX 2

Outstanding challenges in targeting ferroptosis in cancer therapy

Targeting ferroptosis is a highly attractive and powerful therapeutic approach to overcome cancers. However, we are still far away from the clinical horizon. What are the barriers to making ferroptosis inducers into real therapeutics? Here is a list of outstanding questions and challenges that need to be addressed in the ferroptosis field:

- Can we develop ferroptosis-inducing agents, such as compounds and antibodies and nanobodies, without severe side effects? GPX4 inhibitors are preferred candidates, but they also show organ injury and off-target effects because of their chemical properties. Therefore, we need to increase the specificity of ferroptosis inducers.
- Which and what types of cancer are ideal targets for ferroptosis-related cancer therapy? Given that high cell density reduces ferroptosis sensitivity, non-solid tumours may be better targets than solid tumours for ferroptosis induction.
- Can we translate in vivo active ferroptosis inducers, such as compound 28, IKE and icFSP1, into current standard cancer therapy? Several studies indicate that standard chemotherapies such as cisplatin or radiotherapy can synergize with ferroptosis inducers. Thus, we should consider testing these combination soon.
- Can we exploit immune response and anti-tumour immunity for synergistic ferroptosis induction in cancer? Cancer immunotherapy is one of the best options for current cancer treatment as it can enhance ferroptosis sensitivity. Thus, we should consider the combination of cancer immunotherapy and ferroptosis inducers.
- Can we reliably detect ferroptosis in in vivo or in clinical tissue samples after ferroptosis induction? What could be a biomarker for it? Detection of the lipid peroxidation breakdown product (for example, 4-hydroxynonenal) may be one of the best options, but we have to consider that this could also happen in other oxidative stress conditions.

Since RSL3 and erastin were discovered, almost 20 years have passed[3,4]. Moreover, none of their next-generation derivatives have yet entered clinical trials, presumably because these new-generation inhibitors still harbour inappropriate pharmacokinetic properties, lack of specificity, inadequate therapeutic windows and associated side effects. One of the most obvious and attractive targets is GPX4 (Fig. 3). However, GPX4, like GCLC, is essential for embryonic development and is required for tissue homeostasis of a variety of adult tissues and organs, such as the kidney, liver, vasculature and many regions of the brain[87,135]. Therefore, targeting GPX4 in a tumour-specific manner is clearly the preferred route, which could be achieved through a combination of state-of-the-art drug delivery systems or technologies, including ADCs, PROTACs and nanoparticles, and taking advantage of cancer-cell-specific markers for their targeting. Meanwhile, given that mice with knockout of either *Slc7a11* or *Aifm2* are fully viable and do not show any overt phenotypes[20,136], both of these ferroptosis factors could potentially be targeted for ferroptosis-mediated cancer therapy. Nonetheless, there are disadvantages because the use of system x_c^- inhibitors may require high doses that could give rise to off-target effects, and FSP1 inhibitors alone are unlikely to induce cell death. Moreover, additional experimental validation is needed to clarify what types of cancer should be targeted for both pathways. For instance, system x_c^- may be a good target for melanoma because *Slc7a11* knockout can abrogate tumour metastasis[137]. In addition to the challenge of inducing ferroptotic cancer cell death, the relationships between cancer cells and immune cells in the TME needs to be carefully contemplated. It might be highly challenging to induce ferroptosis in cancers while simultaneously preventing ferroptosis in immune cells, such as in neutrophils[121]. Moreover, it remains controversial whether cells that die by ferroptosis indeed mount an anti-tumour immune response[117,119]. Therefore, additional studies are warranted before effective ferroptosis-based anticancer therapies can be realized.

Finally, the occurrence of potential therapy-resistance mechanisms against ferroptosis inducers must be carefully considered when designing new treatment paradigms. For instance, cancer cells can easily acquire ferroptosis resistance through cell–cell contacts[28] and metabolic rewiring, as well as alterations in phospholipid and lipid profiles owing to changes in expression of ACSL4 and its family members[56,119] and other lipid remodelling enzymes, such as LPCAT3, SCD1 and ELOVL fatty acid elongase 5 (ELOVL5)[32,138]. In addition, dietary factors that may influence susceptibility to ferroptosis, such as lipids, vitamin E and selenium, may need to be considered when conducting clinical trials[20,39,139].

Although considerable progress in recent years has helped to determine the molecular mechanisms that underlie ferroptosis, the time is now ripe to take the next steps and advance the field beyond purely detailed mechanistic studies. To that end, the next challenge is to determine which types of cancer cells constitute valid targets in clinical trials and investigate how ferroptosis modulation can be implemented into the current cancer treatment paradigms (see Box 2 for outstanding challenges). Thus, despite remaining uncertainties and important considerations, the question is not if but when the first ferroptosis-based cancer therapy will begin.

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Acknowledgements

The authors would like to apologize to all those colleagues whose work has not been cited here owing to space constraints. M.C. received funding from the Deutsche Forschungsgemeinschaft (DFG) (CO 291/7-1, the Priority Program SPP 2306 (CO 291/9-1, 461385412; CO 291/10-1, 461507177) and the CRC TRR 353 (CO 291/11-1; 471011418), the German Federal Ministry of Education and Research (BMBF) FERROPATH (01EJ2205B) and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement no. GA 884754).

Author contributions

T.N. and M.C. conceived and wrote the manuscript.

Competing interests

M.C. is a co-founder and shareholder of ROSCUE Therapeutics. M.C. and T.N. have filed patents for some of the compounds described herein.

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Peer review information *Nature Cell Biology* thanks the anonymous reviewer(s) for their contribution to the peer review of this work.