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Trends in Molecular Medicine

Opinion

Ferroptosis in the tumor microenvironment: perspectives for immunotherapy

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Targeting ferroptosis, which provokes lipid peroxidation in cancer cells, presents potentially new avenues for anticancer therapy. Recent studies have begun to explore how immune cells in the tumor microenvironment (TME) respond and adapt to lethal lipid peroxides (LPOs). A better understanding of this process in the TME is likely to uncover another side of ferroptosis in cancer immunity and promote the development of ferroptosis-targeted therapy. This Opinion article overviews the main metabolic processes in ferroptosis, summarizes the emerging roles of ferroptosis not only in immune cells in the TME but also in the crosstalk between tumor cells and immune cells, and presents a perspective on the targeting of ferroptosis in cancer immunotherapy.

Highlights

Different immune cells in the TME display distinct sensitivities to ferroptosis.

Ferroptosis is a metabolic vulnerability of activated $CD8^+$ T cells, while inhibition of ferroptosis can promote the survival and antitumor effects of $CD8^+$ T cells in tumors.

In tumors with different immunophenotypes, ferroptosis inducers might have distinct impacts on cancer immunity, which may determine the efficacy of immune checkpoint inhibitor immunotherapy.

Overview of ferroptotic cell death

Ferroptosis, a nonapoptotic form of regulated cell death, plays an important role in cancers and multiple degenerative diseases [1]. Ferroptosis is characterized by the iron-catalyzed accumulation of polyunsaturated fatty acid-containing phospholipids (PUFA-PLs) and peroxidation to lethal levels in cell membranes (Figure 1) [2]. LPOs can induce membrane rupture and increase membrane permeability, leading to cell death [3,4]. **Glutathione (GSH) peroxidase 4 (GPX4)** (see Glossary) is the major detoxifying enzyme that reduces lethal LPOs by using **GSH** as a substrate. **System Xc^-** is an antiporter comprising SLC7A11 and SLC3A2, which imports extracellular cystine in exchange for intracellular glutamate [5]. Once cystine enters cells, it rapidly undergoes reduction, generating cysteine for GSH biosynthesis [6,7]. Some endogenous antioxidants, including reduced coenzyme Q10 (CoQ10) (ubiquinol) and tetrahydrobiopterin (BH4), produced by ferroptosis suppressor protein 1 (FSP1) and GTP cyclohydrolase 1 (GCH1), respectively, also have potent abilities to reduce LPO [8–11].

In recent years, substantial progress has been made in understanding how oncogenic pathways and metabolic reprogramming confer on cancer cells sensitivity to ferroptosis (Box 1) [12]. Inhibition of system Xc^- or of GPX4 is the classic strategy used to induce ferroptosis and has been shown to be efficacious in killing therapy-resistant cancer cells in various studies [13–15]. However, with increasing research on tumor ferroptosis, the inevitable question arises of how immune cells respond to proferroptotic stimulation and how inducers of ferroptosis affect antitumor immunity when used to treat cancers. In this Opinion article, we summarize the emerging roles of ferroptosis in tumor-infiltrating immune cells, and present a perspective on targeting ferroptosis in cancer immunotherapy.

Ferroptosis in tumor-infiltrating immune cells

The **TME** comprises not only cancer cells but also immune cells, including T cells, macrophages, myeloid-derived suppressor cells (MDSCs), dendritic cells (DCs), B cells, and natural killer (NK) cells [16]. Extensive research shows that immune cells in the TME, such as DCs, T cells, and macrophages, share similar growth signals and metabolic properties with cancer

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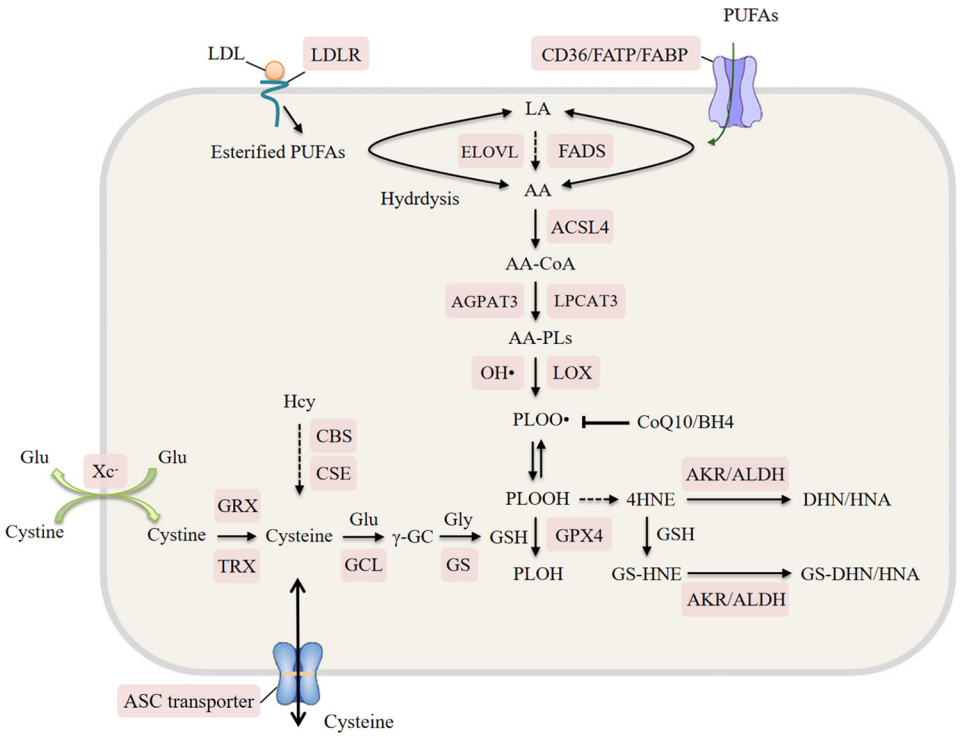

Figure 1. The main metabolic processes in ferroptosis. Human cells acquire polyunsaturated fatty acids (PUFAs) via fatty acid transporter [fatty acid transport protein (FATP), fatty acid-binding protein (FABP), and fatty acid translocase CD36]-mediated uptake of exogenous PUFAs or via low-density lipoprotein (LDL) receptor (LDLR)-mediated uptake of LDLs, the primary carriers of esterified PUFAs. Endogenous PUFA biosynthesis is another, alternative resource, which requires linoleic acid (LA) as a substrate for the elongation of very-long-chain fatty acid protein (ELOVL) and fatty acid desaturase (FADS). Arachidonic acid (AA) is one of the most abundant PUFAs and the most susceptible to lipid peroxidation. Acyl-CoA (AA-CoA) synthetase long chain family member 4 (ACSL4) converts AA into AA-CoA, which is then incorporated into phospholipids by lysophosphatidylcholine acyltransferase 3 (LPCAT3) or 1-acylglycerol-3-phosphate *O*-acyltransferase 3 (AGPAT3). Hydroxyl radicals (OH•) or lipoxygenases (LOXs) initiate the peroxidation of AA-containing phospholipids (AA-PLs), generating lipid radicals and phospholipid hydroperoxides (PLOOHs). Glutathione peroxidase 4 (GPX4) reduces PLOOHs to nontoxic PL-alcohol (PLOH) using glutathione (GSH) as a substrate. Endogenous reduced coenzyme Q10 (CoQ10) (ubiquinol) and tetrahydrobiopterin (BH4) have the ability to scavenge lipid radicals, thus blocking the propagation of lipid peroxidation. PLOOHs can decompose to reactive aldehydes, including 4-hydroxynonenal (4-HNE), which is then metabolized to less-reactive products via aldose reductases (AKRs) or aldehyde dehydrogenases (ALDHs). Extracellular cystine is imported into cells via system Xc^- and is then reduced to cysteine by the GSH/glutaredoxin (GRX) system or the thioredoxin (TRX)/TRX reductase 1 (TRXR1) system. The transsulfuration pathway, comprising cystathionine β-synthase (CBS) and γ-cystathionase (CSE), is responsible for cysteine biosynthesis from homocysteine (Hcy). Cells can also directly absorb or secrete cysteine via the membrane alanine-serine-cysteine preferring (ASC) amino acid transporter. Cysteine and glutamate (Glu) can be ligated by glutamate-cysteine ligase (GCL), producing γ-glutamyl-cysteine (γ-GC). Then, glycine (Gly) is added to γ-GC catalyzed by glutathione synthetase (GS), producing GSH.

cells [17–19]. Undoubtedly, this overlap confers similar vulnerabilities on tumor and immune cells, making it difficult to exploit tumor vulnerability therapeutically without affecting antitumor immunity. Therefore, it is conceivable that inducers of ferroptosis would also provoke lipid peroxidation in immune cells in the TME and affect their functions and viability (Figure 2). The progress regarding ferroptosis in tumor-infiltrating immune cells, including cluster of differentiation 8 $(CD8)^+$ T cells, T regulatory cells (Tregs), macrophages, and MDSCs, as well as indirect evidence linking ferroptosis to NK cells and DCs in the TME is discussed in the following text.

Glossary

15-Hydroperoxyeicosaetetranoic acid (15-HpETE-PE): a PL hydroperoxide, generated by the dioxygenation of AA in phosphatidylethanolamine (PE).
Arachidonate (AA): one of the most abundant but at the same time the PUFA most susceptible to lipid peroxidation in human cells.
$CD8^+$ T cells: critical mediators of antitumor immunity; activated on the detection of tumor-specific antigens.
CD36: a scavenger receptor that functions as a translocase, binding long-chain fatty acids and facilitating their transport into cells.
Damage-associated molecular pattern (DAMP) signals: also known as alarmins; molecules released by stressed cells undergoing necrosis that function as 'find-me' (attractant), 'eat-me' (engulfment), or 'danger' (activation) signals for the recruitment and activation of effector immune cells and exacerbate the inflammatory response.
Glutathione peroxidase 4 (GPX4): the major enzyme against ferroptosis that detoxifies LPOs, even when they are inserted into membranes.
Glutathione (GSH): a tripeptide antioxidant that serves as a cofactor for GPX4 to reduce LPOs.
Immune checkpoint inhibitors (ICIs): monoclonal antibodies that target inhibitory immune checkpoints [e.g., PD-1, cytotoxic lymphocyte antigen 4 (CTLA-4)] to reactivate tumor-specific $CD8^+$ T cells with functional defects due to the presence of immune checkpoint signaling.
Inducible nitric oxide synthase (iNOS): produces NO, an important inflammatory and tumoricidal mediator in macrophages.
Lipoxygenase (LOX)12/15: enzymes that catalyze the dioxygenation of free and esterified PUFAs to generate various lipid hydroperoxides.
Prostaglandin E2 (PGE2): a major oxidative metabolite of AA through the action of the COX pathway and a well-known immunosuppressive factor.
System Xc^-: comprises the heterodimers SLC7A11 and SLC3A2 responsible for importing extracellular cystine in exchange for intracellular glutamate.
Tumor-associated macrophages (TAMs): one of the most abundant immune cells in the TME. TAMs can

Box 1. Regulators of ferroptosis in immune cells

Ferroptosis is orchestrated by complex metabolic and molecular pathways, which can be divided into three major parts: iron-dependent peroxidation of polyunsaturated PLs, LPO scavenging, and the repair of damaged plasma membranes. Metabolic pathways that regulate the availability of free iron (e.g., autophagy, NRF2), hydrogen peroxide [e.g., cytochrome P450 reductase (POR)], PUFAs [e.g., lipophagy, ELOVL, adenosine 5′-monophosphate-activated protein kinase (AMPK)], and PUFA-enriched PLs (e.g., ACSL4) control ferroptosis sensitivity [51,70–72]. The major protective mechanisms that eliminate LPOs involve the system Xc^-/cysteine/GSH/GPX4, cytosolic CoQ10/FSP1, mitochondrial CoQ10/dihydroorotate dehydrogenase (DHODH), and GCH1/BH4 axes [8,73]. Many molecules – such as NRF2, breast cancer gene 1 (BRCA1)-associated protein 1 (BAP1), lncRNA, etc. – can regulate the activities of the defense systems via transcriptional or post-transcriptional mechanisms [51,74]. Accumulation of LPOs can induce membrane rupture and ferroptotic cell death, while the endosomal sorting complex required for transport III (ESCRT-III) plays a key role in the repair of damaged plasma membranes and limits ferroptosis [3,75].

Regulators of ferroptosis have been investigated rarely in immune cells, among which system Xc^-/SLC7A11 and GPX4 have been studied relatively more extensively. Naïve T cells are unable to generate cysteine individually due to the lack of SLC7A11 and the low activity of the transsulfuration pathway [76,77]. Instead, they can directly import the cysteine produced by DCs and macrophages via the alanine-serine-cysteine preferring (ASC) amino acid transporter [78–81]. Nonetheless, the expression of SLC7A11 in activated T cells is highly induced [76,82]. SLC7A11 level and cystine availability have been shown to be required for T cell expansion *in vitro* [83]. However, deletion of SLC7A11 or cystine deprivation *in vivo* did not reduce the viability of T cells and did not affect the antitumor immune function of T cells. The activity of SLC7A11 and cystine uptake were also reported to be strongly induced in M1 macrophages [84]. Deletion of SLC7A11 greatly reduced the survival of M1 macrophages at the inflammatory site [85,86]. Notably, SLC7A11 was reported to be required for iNOS-dependent NO• production. Deprivation of cyst(e)ine instead of GSH can significantly decrease NO• production in M1 macrophages [87]. Therefore, M1 macrophages might be more vulnerable to system Xc^- inhibitors than to GPX4 inhibitors, given that the NO•-dependent ferroptosis-protective mechanism is suppressed by the system Xc^- inhibitors. It should be noted that targeting of SLC7A11 and GPX4 might also play a ferroptosis-independent role in other types of cell death (apoptosis, pyroptosis, and necroptosis) [88]. While exploring ferroptosis in immune cells by targeting GPX4 or SLC7A11, it will be necessary to design more complex experiments to distinguish the ferroptosis-independent role in GPX4 or SLC7A11 inhibition.

polarize into the antitumor M1 phenotype and the tumor-promoting M2 phenotype in response to different stimuli.

Tumor microenvironment (TME): a complex biological network whose diverse components, including tumor cells, mesenchymal cells, immune cells, and the surrounding matrix, influence the hallmarks and fates of tumor cells.

X-box binding protein-1 (XBP1): the endoplasmic reticulum (ER) stress response transcription factor that activates unfolded protein response (UPR) target genes via direct binding to the UPR element.

Antitumor T cells are sensitive to ferroptosis

T cells are critical mediators of antitumor immunity [18]. It was previously reported that T cells lacking GPX4 rapidly accumulated membrane LPO after activation and concomitantly underwent ferroptosis, failing to expand and prevent infection in an *in vivo* infection model [20]. However, the influence of GPX4 deletion on tumor-infiltrating T cells was not explored in this study. A recent study showed that large amounts of LPO were detectable in **$CD8^+$ T cells** derived from tumors but not from lymph nodes and identified ferroptosis as a metabolic vulnerability of tumor-specific $CD8^+$ T cells [21]. Comparison of co-cultured T cell receptor (TCR)-transgenic $CD8^+$ T cells and antigen-expressing cancer cells revealed that activated $CD8^+$ T cells were relatively more sensitive to GPX4 inhibitors. The specific killing rates and numbers of $CD8^+$ T cells were reduced by GPX4 inhibitors at concentrations that had no impact on the survival of the cancer cells. Conventional $CD4^+$ T cells (Tconvs) exhibited sensitivity to GPX4 inhibitors similar to that of $CD8^+$ T cells after activation [21].

The mechanisms that determine susceptibility to ferroptosis in tumor cells have been researched extensively over the past years, but similar studies on T cells are still scarce (Box 1). One such study confirmed a crucial role of acyl-CoA synthetase long chain family member 4 (ACSL4) in dictating activated $CD8^+$ T cell sensitivity to ferroptosis; deficiency of ACSL4 was shown to potentially prevent ferroptosis induced by GPX4 inhibitors in these cells [21]. Further studies reported that fatty acid translocase **CD36**-expressing $CD8^+$ T cells underwent ferroptosis and displayed lower interferon gamma (IFNγ) production in the TME with abundant fatty acids or oxidized lipids. CD36 expression was reported to be required for fatty acid- or oxidized lipid-induced ferroptosis and impaired the cells' cytotoxic cytokine production and antitumor function *in vitro* and *in vivo* [22,23].

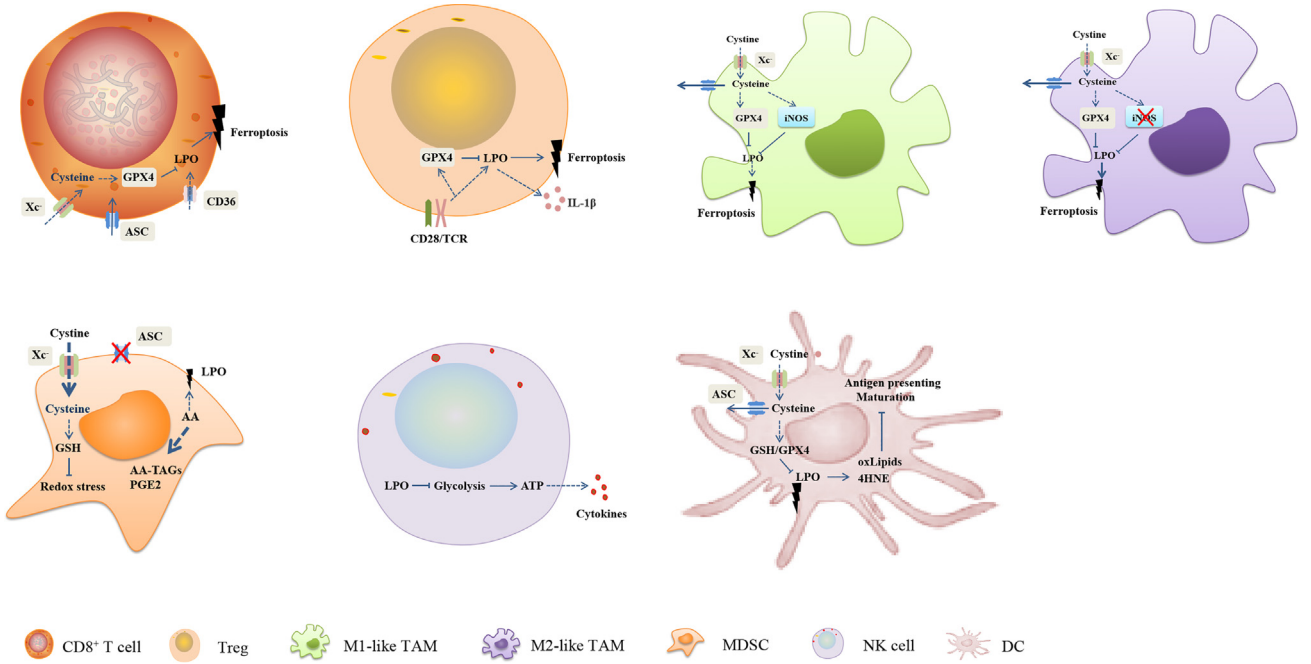

Figure 2. Sensitivity of immune cells in the tumor microenvironment (TME) to ferroptosis. Activated CD8$^+$ T cells display large amounts of lipid peroxides (LPOs) due to the expression of fatty acid translocase CD36 and are sensitive to ferroptosis induced by glutathione peroxidase 4 (GPX4) inhibitors. On inhibition of system Xc$^-$, CD8$^+$ T cells in the TME survive dependent on exogenous cysteine (Box 1), transported by the alanine-serine-cysteine preferring (ASC) transporter. T regulatory cells (Tregs) induce the expression of GPX4 after activation by T cell receptor (TCR)/CD28 co-stimulation. Deletion of GPX4 in Tregs led to ferroptosis and promoted the production of IL-1β, thus potentiating antitumor immunity. M1 tumor-associated macrophages (TAMs) exhibit high resistance to ferroptosis induced by the deletion of GPX4, due to the high levels of inducible nitric oxide synthase (iNOS) and nitric oxide radical (NO•) production. Targeting of system Xc$^-$ can reduce the survival of M1-like TAMs, as cyst(e)ine is also required for iNOS-dependent NO• production. M2-like TAMs can be killed and/or repolarized into the M1 phenotype by ferroptosis inducers due to suppressed expression of iNOS. Myeloid-derived suppressor cells (MDSCs) selectively accumulate arachidonate (AA)-esterified triglycerides (AA-TAGs) and prostaglandin E2 (PGE2) but not AA-containing LPOs, contributing to their resistance to ferroptosis. They also express high levels of system Xc$^-$ with intensive cystine uptake but do not transport cysteine due to the absence of the ASC transporter. There are no direct studies of ferroptosis in tumor-associated natural killer (NK) cells and dendritic cells (DCs). NK cells in the TME exhibited increased expression of proteins involved in lipid peroxidation and had a cellular morphology similar to that of ferroptotic cells. Lipid peroxidation-associated oxidative stress suppressed NK cell glucose metabolism, resulting in NK cell dysfunction in the TME. Targeting of GPX4 or system Xc$^-$ can cripple the maturation process of naïve DCs and normal DC function by inducing the generation of 4-hydroxynonenal (4-HNE) and lipid oxidation products.

Overexpression of GPX4 can protect CD8$^+$ T cells from ferroptosis induced by GPX4 inhibitors or lipids and restore the cells' cytotoxic cytokine production *in vitro* [21,23]. *In vivo*, GPX4 overexpression in CD8$^+$ T cells can increase the number of tumor-infiltrating CD8$^+$ T cells and lead to enhanced tumor control [22,23]. The recent studies have also investigated the effects of ferroptosis inducers or inhibitors on T cell-mediated antitumor immunity. GPX4 inhibitors can promote the survival of cancer cells via selective killing of CD8$^+$ T cells in an *in vitro* co-culture system, while the ferroptosis inhibitor ferrostatin-1 rescued T cell-mediated specific killing of cancer cells [21]. Tumor-bearing mice with adoptive transfer of ferrostatin-1-treated CD8$^+$ T cells displayed smaller tumor burden and better survival, while GPX4 inhibitor-treated CD8$^+$ T cells exerted an impaired antitumor effect in the mice [22]. These studies implied that ferroptosis inhibitors might be a promising strategy to enhance antitumor immunity, although the speculations were drawn only from *in vitro* experiments and *in vivo* experiments with non-systemic administration.

However, these results contradicted a previous study, which suggested that tumor cells exhibited higher sensitivity to ferroptosis than activated CD8$^+$ T cells and that ferroptosis inducers do not impair the antitumor activities of activated CD8$^+$ T cells *in vitro* and *in vivo* [24]. This conflicting

finding might be due to the different methods of inducing ferroptosis. In this study, a system Xc^- inhibitor and cyst(e)inase, an enzyme that degrades cystine and cysteine, were used to induce ferroptosis in the majority of experiments, especially *in vivo*, while $CD8^+$ T cells might still survive via an alternative cysteine (Figure 1 and Box 1) [24]. It should be noted that, in this study, the ferroptosis inhibitor liproxstatin-1 alone exhibited no effect on tumor growth after systemic administration and even reversed the effect of immunotherapy by preventing IFNγ-induced ferroptosis in tumor cells [24]. The effect of ferroptosis inhibitors on tumor growth and immunotherapy might depend on the differences in sensitivity to proferroptotic stimulation between tumor cells and $CD8^+$ T cells. Therefore, there needs to be more research applying ferroptosis inhibitors in different tumors under different conditions and exploring their effect on immunotherapy.

GPX4 prevents ferroptosis to sustain activated Treg survival

Tregs are a portion of $CD4^+$ T cells and repress antitumor immunity [25]. Compared with tumor-specific $CD8^+$ T cells, tumor-derived Tregs displayed low amounts of LPO, indicating that few Tregs undergo ferroptosis in the TME [21]. This might be because Tregs rapidly induce the expression of GPX4 after activation by TCR/CD28 co-stimulation [26]. GPX4 can protect activated Tregs from aberrant lipid peroxidation and ferroptosis but is dispensable for the survival of Tregs at steady state. Genetic deletion of GPX4 in Tregs led to ferroptosis, not other types of cell death in activated Tregs, and promoted the production of IL-1β that facilitated T helper 17 (Th17) cell response, thus potentiating antitumor immunity and repressing tumor growth. Ferroptosis inhibitors rescued the ferroptosis of activated GPX4-deficient Tregs and restored their immunosuppressive function *in vitro* and in tumors. System Xc^- inhibitors rarely impaired Treg viability [26]. These results indicate that inducing ferroptosis in Tregs by targeting GPX4 may be a therapeutic strategy for cancer treatment. However, this study did not investigate the effect of systemic administration of GPX4 inhibitors on tumor immunity and tumor growth.

M1 macrophages exhibit higher resistance to ferroptosis than M2 phenotypes

Macrophages in the TME are normally referred to as **tumor-associated macrophages (TAMs)**. Resting macrophages can polarize into two distinct activation states, termed M1 and M2 phenotypes. In general, TAMs predominantly exhibit the M2-like phenotype, suppressing antitumor immunity [27]. Although the amount of LPO and levels of expression of ACSL4, lysophosphatidylcholine acyltransferase 3 (LPCAT3), and GPX4 were similar in M1 and M2 macrophages, the M1 subtype exhibited higher resistance to ferroptosis induced by deletion of GPX4 [28]. Mechanistically, this can be explained by high levels of **inducible nitric oxide (NO) synthase (iNOS)** and NO radical (NO•) production in M1 cells, while iNOS expression and NO• production are suppressed in M2 and resting macrophages. NO• can readily react with the lipid radicals and the reactive intermediates derived from lipid peroxidation, substituting GPX4 as an antiferroptotic defense. Although these findings are drawn from *in vitro* experiments and an infection model, the researchers also assessed the role of iNOS/NO• in the regulation of macrophage survival in an *in vivo* tumor model [28]. The results showed that deletion of iNOS in bone marrow-derived macrophages had no impact on the population of M2 TAMs in the TME but significantly reduced the population of M1 TAMs; this suggests that M1 TAMs without iNOS cannot adapt to the proferroptotic TME, particularly when these cells need to phagocytose ferroptotic cells with accumulating LPO [28,29].

Elimination of M2 TAMs or repolarization of the M2 phenotype into the antitumor M1 phenotype has gained popularity in the field of cancer immunity [30]. Evidence suggests that targeting these cells with ferroptosis inducers is a potentially promising therapeutic strategy to reverse the immunosuppressive TME. Deletion of GPX4 in TAMs can inhibit the survival of M2 TAMs while leaving the population of M1 TAMs unaffected [28]. M2 TAMs can be efficiently repolarized into the M1

phenotype by proferroptotic nanoparticles [31,32]. However, GPX4 might be indispensable for macrophages to initiate their innate immune responses [33]. Hence, it will be important to investigate the impact of ferroptosis inducers on the viability and functions of macrophages in the TME.

MDSCs exhibit resistance to ferroptosis

MDSCs are present in most tumors and have potent immunosuppressive capacity in the TME [34]. Evidence indicates that these tumor-infiltrating MDSCs are protected from ferroptosis by the expression of high levels of system Xc^- and the neutral ceramidase *N*-acylsphingosine amidohydrolase (ASAH2), which destabilizes p53 protein [35,36]. Moreover, liquid chromatography–mass spectrometry (LC-MS) analysis showed that a subset of MDSCs in the TME selectively accumulate **arachidonate (AA)**-esterified triglycerides (AA-TAGs), oxidized AA-TAGs, and prostaglandin E2 (PGE2), a major oxidative metabolite of free AA but not AA-PLs and related LPOs. This implies that the metabolic conversion of AA to AA-PLs and peroxidation might be suppressed in tumor-infiltrating MDSCs, contributing to their resistance to ferroptosis [37,38].

Evidence linking ferroptosis to NK cells and DCs in the TME

NK cells are central to antitumor immunity and defects in NK cell function lead to higher rates of tumorigenesis and tumor growth [39]. A recent study showed that tumor-associated NK cells exhibited increased expression of proteins involved in ferroptosis, lipid peroxidation, and oxidative damage, and had a cellular morphology similar to that of ferroptotic cells [40]. Lipid peroxidation-associated oxidative stress suppressed NK cell glucose metabolism, resulting in NK cell dysfunction in the TME. Activation of nuclear factor E2-related factor 2 (NRF2), a critical factor in mitigating ferroptosis, rescued NK cell glucose metabolism and antitumor activity *in vivo* [40,41]. However, this study did not explore the effect of ferroptosis inducers and inhibitors on the survival and function of tumor-associated NK cells.

DCs are professional antigen-presenting cells required for naïve T cell activation and sustaining T cell-dependent immunity [42]. Indirect evidence suggests that DC function is also influenced by ferroptosis. Tumor-associated DCs often display a reduced capacity to process antigens due to increased levels of lipids, a feature associated with vulnerability to ferroptosis [43–45]. Increased levels of 4-hydroxynonenal (4-HNE)-protein adducts, a byproduct of lipid peroxidation, have been reported in tumor-associated DCs; this could trigger constitutive **X-box binding protein-1 (XBP1)** activation and malfunction of DCs [46]. Bone marrow-derived naïve DCs exhibit high levels of **lipoxygenase (LOX)12/15** during the maturation process, producing abundant PL oxidation products, which in turn restrict the maturation of DCs [47]. Therefore, it is possible that targeting GPX4 or system Xc^- might cripple the maturation process of naïve DCs and normal DC function in the TME.

Tumor-infiltrating B cells mainly originate from memory B cells and comprise different subpopulations with opposite functions in tumor immunity [48,49]. Memory B cells are differentiated from conventional B cells (B2 cells). To date, there is no study showing the relationship between ferroptosis and tumor-infiltrating B cells. A previous study showed that GPX4 is critical for the survival of B1 and marginal zone (MZ) B cells but not follicular B2 cells. B1 and MZ B cells expressed higher levels of CD36 and took up higher levels of lipids than follicular B2 cells. GPX4 deletion evoked lipid peroxidation and ferroptosis of B1 and MZ B cells but not follicular B2 cells [50]. This study indicates an interesting research direction to explore the effect of proferroptotic stimulation on the survival of tumor-infiltrating B cells and B cell-mediated tumor immunity.

In conclusion, existing research has indicated the dual role of ferroptosis in tumor immunity. On the one hand, inducing ferroptosis might suppress the survival of antitumor immune cells

(including $CD8^+$ cells, NK cells, and DCs) and lead to functional defects. On the other hand, some immunosuppressive immune cells, such as M2 TAMs and Tregs, also need GPX4 and potentially other factors to prevent ferroptosis and sustain cell activation. Induction of ferroptosis in these cells might lead to cell death and reverse their protumor function.

Ferroptosis-mediated crosstalk in the TME

Increased immunogenicity of ferroptotic cancer cells

Ferroptotic cancer cells can release some 'find-me' and 'eat-me' immunostimulating signals, notably **damage-associated molecular pattern (DAMPs) signals** (e.g., HMGB1, calreticulin, DNA, ATP) [51,52], which allow DCs, macrophages, and other immune cells to properly locate the site of dying tumor cells [12]. Recently, a membrane oxidized PL, 1-steaoryl-2-15-HpETE-sn-glycero-3-PE, was identified to be a crucial 'eat-me' signal on the ferroptotic cell surface that leads to phagocytosis by macrophages [29]. The increased immunogenicity of ferroptotic cancer cells was shown to induce tumor-specific immune responses, enhancing the efficacy of **immune checkpoint inhibitor (ICI)** and anti-programmed cell death protein 1 (PD-1)/PD-L1 therapy [53,54]. Additionally, early ferroptotic cancer cells can promote the phenotypic maturation of DCs and elicit a vaccination-like effect [52].

Immunosuppressive signals from ferroptotic tumor cells

While ferroptotic tumor cells are immunostimulatory, they can also act through multiple mechanisms to suppress antitumor immune responses. Proferroptotic stimulation can promote the release of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a major product of oxidative DNA damage, from cancer cells. Released 8-OHdG activates the STING-dependent DNA sensor pathway of TAMs, which results in infiltration by TAMs and M2 polarization, thus promoting pancreatic tumorigenesis [55]. Moreover, the oncogenic Kirsten rat sarcoma viral oncogene homolog (KRAS) protein in exosomes released from ferroptotic tumor cells can be taken up by TAMs. This, in turn, causes TAMs to switch to an M2 phenotype, thereby promoting pancreatic tumor growth [56].

Ferroptotic cancer cells are associated with the release of PGE2, a key immunosuppressive factor that suppresses the antitumor functions of NK cells, DCs, and cytotoxic T cells [57,58]. Ferroptotic cancer cells can also release many types of oxidized PLs [e.g., **15-hydroperoxyeicosaetetranoic acid (15-HpETE-PE)**, 15-HETE-PE] and oxidatively truncated species (oxidized PLs with subsequent shortening of the oxygenated PUFA residues), oxidized eicosanoids (generated by direct oxidation of PUFAs; e.g., 5-HpETE), 4-HNE, and their derivatives [12,55,57,59]. Little information exists on the functions of these lipid-derived products. However, they might limit the maturation of DCs and antigen cross-presentation [47,60]. 15-HpETE-PE is a well-defined proferroptotic lipid. High levels of 15-HpETE-PE and other LPO species can be released into the TME by ferroptotic tumor cells after disintegration or before death. Epithelial cells and immune cells exposed to 15-HpETE-PE will rapidly undergo ferroptosis [28,61].

Immune cells regulate tumor cell ferroptosis

Activated $CD8^+$ T cells can provoke lipid peroxidation and induce ferroptosis in tumor cells, contributing to their antitumor effects [24]. Mechanistically, IFNγ, released from activated $CD8^+$ T cells, inhibits the expression of system Xc^- by activating the JAK–STAT1 pathway in tumor cells, thus sensitizing tumor cells to proferroptotic stimulation, including GPX4 or system Xc^- inhibitors, and cystine deprivation. This indicates that $CD8^+$ T cell-mediated ferroptosis in tumor cells is an important, previously undefined mechanism that contributes to the antitumor efficacy of T cells and ICI immunotherapy. However, on proferroptotic stimulation by IFNγ, tumor cells might also increase the release of immunosuppressive signals to suppress T cell survival and promote infiltration by immunosuppressive immune cells, acting as a feedback protective

mechanism. Therefore, it will be important to weigh the pros and cons of IFNγ-induced ferroptosis. We speculate that, depending on the circumstances, the ferroptosis-based balance between immune evasion and immune elimination in tumors might shift distinctly and directly affect the efficacy of ICI immunotherapy. When the balance shifts towards ferroptosis-mediated immunosuppression, IFNγ-induced tumor cell ferroptosis might cripple antitumor immunity and even result in acquired tolerance to immunotherapy. Collectively, the available findings imply that there exists a complicated ferroptosis-based crosstalk between tumor cells and immune cells.

Perspectives on targeting ferroptosis in cancer immunotherapy

The TME can be divided into three immunophenotypes: inflamed type, immune-altered type, and immune-desert type, based on the distribution of infiltrating immune cells [62,63]. Inflamed tumors are associated with high immunogenicity, intact antigen presentation, and abundant $CD8^+$ T cell infiltration. Immune-altered tumors are characterized by an immune-suppressive TME

Key figure

Speculative combination immunotherapy strategies based on the tumor microenvironment (TME) immunophenotypes and currently available information on the sensitivity of immune cells to ferroptosis

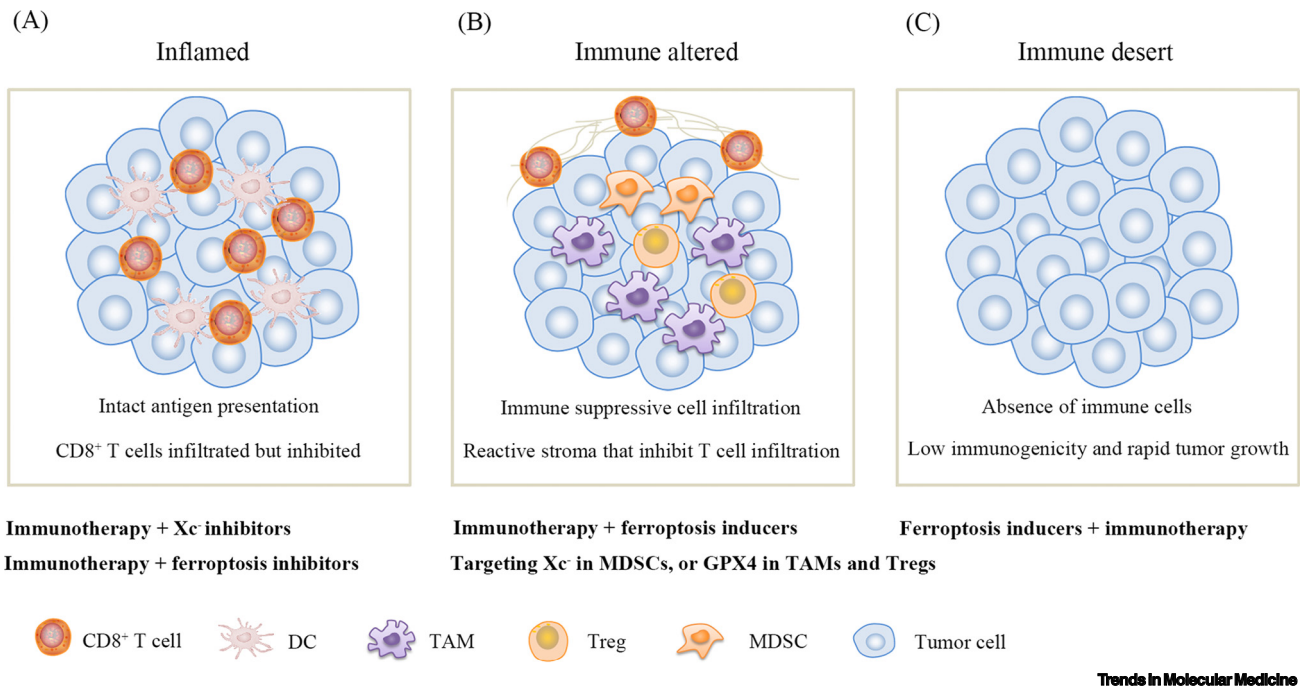

Figure 3. (A) Inflamed tumors are associated with high immunogenicity, intact antigen presentation, and abundant $CD8^+$ T cell infiltration. Ferroptosis inducers might dramatically kill the abundant infiltrated $CD8^+$ (and $CD4^+$) T cells and cripple the maturation process of naïve dendritic cells (DCs) and normal DC function, thereby reducing the efficacy of immune checkpoint inhibitor (ICI) immunotherapy. Targeting of system Xc^- will not reduce the viability of T cells. In some cases, ferroptosis inhibitors might be used to protect T cells from ferroptosis due to the proferroptotic TME and block the release of immunosuppressive signals from proferroptotic tumor cells. (B) Immune-altered tumors are characterized by high levels of infiltrated myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), or T regulatory cells (Tregs). Glutathione peroxidase 4 (GPX4) inhibitors might be applied to eliminate M2 TAMs and Tregs, while targeting of system Xc^- could alleviate MDSC-mediated cystine deprivation in the TME, thus promoting T cell survival. (C) Immune-desert tumors exhibit rapid tumor growth and lack immune cell infiltration due to the low immunogenicity. These tumors tend to respond poorly to immunotherapy, and targeted drugs or chemoradiotherapy may have better efficacy. However, some targeted drugs and chemoradiotherapy are also ferroptosis inducers, which may increase the immunogenicity of tumor cells and enhance the efficacy of combined immunotherapy.

due to the high levels of infiltrating MDSCs, TAMs, or Tregs. Immune-desert tumors lack immune cell infiltration, probably due to the low immunogenicity or the presence of metabolic barriers in the TME.

Although ferroptosis inducers have been reported to enhance the efficacy of ICI immunotherapy in several studies [24,53,54], this combined treatment strategy is not necessarily suitable for all conditions. In tumors with different immunophenotypes, the impact of ferroptosis inducers on the tumor immunity is likely to be distinct (Figure 3, Key figure). In addition, immune cells exhibit distinct responses to different kinds of ferroptosis inducers, such as GPX4 inhibitors and system Xc^- inhibitors (Box 1). Choosing the specific type of ferroptosis inducers to combine with ICI immunotherapy is thus a matter that needs to be considered.

The immune advantage of ferroptosis inducers might be minimal due to the inherent immunogenicity in inflamed tumors. However, ferroptosis inducers could dramatically kill the abundant infiltrating $CD8^+$ and $CD4^+$ T cells and cripple the maturation process of naïve DCs and normal DC function, thereby reducing the efficacy of ICI immunotherapy (Figure 3).

Immunosuppressive TAMs are sensitive to ferroptosis and can be repolarized into the M1-like TAMs; therefore, in immune-altered tumors with high levels of TAM infiltration, ferroptosis inducers may potentially reverse the immunosuppressive TME and primary ICI immunotherapy resistance. Inducing ferroptosis in Tregs by GPX4 inhibitors can suppress the survival of activated Tregs and promote a Th17 cell response, thus potentially potentiating antitumor immunity and the efficacy of immunotherapy in tumors with high levels of Treg infiltration. However, in tumors with high levels of MDSC infiltration, ferroptosis inducers might not be a good choice, as MDSCs are suggested to be resistant to ferroptosis [35,36]. MDSCs express high levels of system Xc^- with intensive cystine uptake but do not transport cysteine due to the absence of the ASC transporter. They can compete for cystine with other immune cells and do not return cysteine to the TME, thereby depriving T cells of cyst(e)ine [36]. From this point of view, targeting system Xc^- in tumors might alleviate MDSC-mediated cystine deprivation in the TME, thus promoting T cell survival (Figure 3).

Immune-desert tumors exhibit rapid tumor growth and lack immune cell infiltration due to the low immunogenicity. This kind of tumor tends to respond poorly to immunotherapy, and chemoradiotherapy or targeted drugs may have a better efficacy [62]. However, some targeted drugs (e.g., sorafenib), chemotherapy (e.g., cisplatin), and radiotherapy are also ferroptosis inducers [64–66]. These treatments may increase the immunogenicity of tumor cells and promote immune cell infiltration, which might enhance the efficacy of ICI immunotherapy. In clinical practice, targeted therapy, radiotherapy, and chemotherapy have already been applied in combination with immunotherapy in various tumors [67–69]. It would be interesting to illuminate whether chemoradiotherapy or targeted drugs with proferroptotic properties are more effective in enhancing the efficacy of ICI immunotherapy.

The TME is a proferroptotic environment, with fatty acids, redox stress, cyst(e)ine competition, and other proferroptotic factors, leading to $CD8^+$ T cell vulnerability to ferroptosis [22]. For example, MDSCs have been reported to inhibit T cell activation by depleting cyst(e)ine in the TME [36]. Therefore, in some cases, ferroptosis inhibitors might be used to protect T cells from ferroptosis and block the release of immunosuppressive signals from preferroptotic tumor cells, thus enhancing the efficacy of ICI immunotherapy (Figure 3). This speculation is supported by the findings that the ferroptosis inhibitor ferrostatin-1 promoted higher IFNγ production and proliferation of $CD8^+$ T cells in tumors, and prevention of $CD8^+$ T cell ferroptosis by targeting CD36 enhanced the efficacy of ICI immunotherapy [22]. The combined effect of ferrostatin-1 and ICI immunotherapy, however, was not investigated in this study. Further research is needed to

Clinician's corner

Targeting of ferroptosis is efficacious in killing standard therapy-resistant cancer, such as human epidermal growth factor receptor (EGFR)2 (HER2)-amplified breast cancer and EGFR-mutant non-small cell lung cancer, in preclinical studies [14].

Some regulators and metabolites related to iron metabolism, energy metabolism, lipid metabolism, and redox metabolism, such as the transferrin receptor (TFRC), and ACSL4, hypoxia-inducible factor 2 (HIF2), confer on cells sensitivity to ferroptosis, which might act as a biomarker to predict a ferroptotic response [44]. However, ferroptosis in tumors might not be well predicted by only a single biomarker.

In preclinical studies, some ferroptosis inducers can increase the immunogenicity of cancer cells to induce tumor-specific immune responses, strengthening ICI immunotherapy efficacy in some tumors such as prostate cancer and glioma [53,54].

Early ferroptotic cancer cells can promote the phenotypic maturation of bone marrow-derived DCs and elicit a vaccination-like effect [52].

Induction of ferroptosis might suppress the survival of antitumor immune cells, such as $CD8^+$ cells and NK cells, and lead to defects in antitumor immunity, which might attenuate the efficacy of ICI immunotherapy, particularly in inflamed tumors.

Under physiological conditions, tumor-infiltrating immune cells also undergo ferroptosis due to the proferroptotic TME. In some cases, inhibition of ferroptosis can be used to promote the survival of $CD8^+$ T cells and enhance their antitumor function [22].

explore more factors in the TME that contribute to the ferroptosis of $CD8^+$ T cells and ascertain whether and how tumor and immunosuppressive cells promote ferroptosis in $CD8^+$ T cells. It should also be elucidated whether ICI immunotherapy sensitizes $CD8^+$ T cells to the proferroptotic TME during their reactivation.

Concluding remarks

Available evidence, albeit currently limited, demonstrates distinct responses of immune cells to ferroptosis inducers and complicated ferroptosis-based crosstalk between tumor cells and immune cells. It will be important to elucidate the regulation of cancer immunity by proferroptotic therapy, including the system Xc^-/GPX4 inhibitors, immunotherapy, and chemoradiotherapy, as well as by the proferroptotic factors in the TME, including cyst(e)ine competition, certain metabolites, and redox stress. While studies have highlighted the promising role of ferroptosis inducers in strengthening ICI immunotherapy efficacy, further research is necessary to establish when to apply ferroptosis inducers to increase the immunogenicity of tumor cells or to kill immunosuppressive cells and when to apply ferroptosis inhibitors to protect antitumor immune cells from ferroptosis (see Outstanding questions). Clarifying these questions will aid the development of ferroptosis-based combined immunotherapy for certain tumors.

Outstanding questions

How do specific immune cells respond to proferroptotic stimulation?

How do different inducers of ferroptosis affect antitumor immunity when used to treat cancers?

In which tumor types will ferroptosis inducers in combination with ICI immunotherapy be an effective cancer treatment?

What are the factors in the TME that contribute to the ferroptosis of $CD8^+$ T cells and other immune cells?

Will ICI immunotherapy sensitize $CD8^+$ T cells to the proferroptotic TME during their reactivation?

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Declaration of interests

The authors have no interests to declare.

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