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REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

Ferroptosis: Biology and Role in Gastrointestinal Disease

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Ferroptosis is a form of nonapoptotic cell death that involves iron-dependent phospholipid peroxidation induced by accumulation of reactive oxygen species, and results in plasma membrane damage and the release of damage-associated molecular patterns. Ferroptosis has been implicated in aging and immunity, as well as disease states including intestinal and liver conditions and cancer. To date, several ferroptosis-associated genes and pathways have been implicated in liver disease. Although ferroptotic cell death is associated with dysfunction of the intestinal epithelium, the underlying molecular basis is poorly understood. As the mechanisms regulating ferroptosis become further elucidated, there is clear potential to use ferroptosis to achieve therapeutic benefit.

Keywords: Cancer; Inflammation; Tumor Microenvironment; Cell Death.

Ferroptosis is an iron-dependent mechanism of nonapoptotic cell death caused by phospholipid (PL) peroxidation. As a distinct form of regulated cell death (RCD),[1–3] ferroptosis has gained much attention in recent years due to its involvement in pathologic processes and immunity including cancer and its related therapy. Ferroptosis initiators have been broadly classified as extrinsic resulting from altering the activity of cell membrane amino acid transporters and iron transporters, or as intrinsic due to inhibition of intracellular antioxidant enzymes such as glutathione peroxidase 4 (GPX4).[4] Ferroptosis is regulated not by a single universal mechanism, but by diverse and context-dependent pathways that converge at the accumulation of lipid peroxides in the plasma membrane.[5] Unlike other forms of RCD, such as apoptosis (caspases), necroptosis (mixed-lineage kinase domain–like pseudokinase) or pyroptosis (gasdermin D), specific and indispensable mediators for the execution of ferroptosis remain largely unknown, although proposed models exist.[6,7] Propagation of ferroptosis through the release of damage-associated molecular patterns (DAMPs) has been shown.[8–10] DAMPs are immuno-modulatory and can connect ferroptosis with the tumor microenvironment (TME) with implications for immunotherapy as cancer treatment. Of much clinical relevance is how to therapeutically target ferroptosis in a disease context. In this review, we discuss mechanisms and regulation of ferroptosis including its relevance in diseases of the gut and cancer. Furthermore, regulatory axes of ferroptosis and their targeting represent a therapeutic opportunity in cancer cells that have developed acquired resistance to other forms of RCD.

Ferroptosis Regulation

Key pathways that can trigger ferroptotic cell death converge in the peroxidation of membrane lipids. One such pathway involves the degradation of ferritin and the engagement of transferrin receptor (TFRC)-mediated iron import, both of which serve to increase the intracellular iron pool leading to lipid peroxidation. Through the Fenton reaction, this iron pool and lipid metabolizing enzymes (ACSL4 and LPCAT3) contribute to an increase in PL peroxidation that leads to ferroptosis. GPX4 is the canonical ferroptosis-controlling pathway for the regulation of ferroptosis. The nuclear factor erythroid 2-related 2 (NRF2) transcription factor regulates GPX4[11] and SCL7A11,[12] as well as other lipid peroxidation and ferroptosis-related genes.[13] Initiators of ferroptosis that act through the GPX4-dependent pathway include depletion of glutathione (GSH; the reducing substrate of GPX4 activity), inhibition of

Abbreviations used in this paper: **AA, arachidonic acid; ACLF, acute-on-chronic liver failure; ALD, alcoholic liver disease; AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; BH_4, tetrahydrobiopterin; CAC, colitis-associated carcinogenesis; CD, Crohn's disease; CDI, cysteine-deprivation-induced; CoA, coenzyme A; CoQ10, coenzyme Q10; CRC, colorectal cancer; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; DECR1, 2,4-dienoyl-CoA reductase 1; DHODH, dihydroorotate dehydrogenase; DPP4, dipeptidyl peptidase-4; DSS, dextran sulphate sodium; ELAVL1, ELAV-like protein 1; EMT, epithelial-mesenchymal transition; ER, endoplasmic reticulum; FADS2, fatty acid desaturase 2; FSP1, ferroptosis suppressor protein 1; GI, gastrointestinal; GPX4, glutathione peroxidase 4; GSH, glutathione; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HO1, heme oxygenase 1; HSCs, hepatic stellate cells; IBD, inflammatory bowel disease; ICB, immune checkpoint blockade; IECs, intestinal epithelial cells; IFN-γ, interferon gamma; IL, interleukin; IR, ionizing radiation; MBOAT1/2, Membrane Bound O-Acyltransferase Domain Containing 1 and 2; MDSC, myeloid-derived suppressor cell; MUFA, monounsaturated fatty acid; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; mTOR, mammalian target of rapamycin; MTORC1, mammalian target of rapamycin complex 1; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NF-κB, nuclear factor kappa B; NRF2, nuclear factor erythroid 2-related 2; PE, phosphatidyl ethanolamine; PGE2, prostaglandin E2; PL, phospholipid; PMN, polymorphonuclear; PUFA, polyunsaturated fatty acid; RCD, regulated cell death; ROS, reactive oxygen species; SCFA, short-chain fatty acid; SIRT1, sirtuin 1; TAMs, tumor associated macrophages; TCA, tricarboxylic acid cycle; TFRC, transferrin receptor; TME, tumor microenvironment; TNF, tumor necrosis factor; Tregs, regulatory T cells; UC, ulcerative colitis; ZFP36, zinc finger protein 36.**

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the cysteine importer known as system X_c^- using erastin,[14] or GPX4 inhibition via the small molecule RSL3.[15] There are also GPX4-independent pathways that serve as alternative antioxidant defenses against ferroptosis, such as the ferroptosis suppressor protein 1 (FSP1), dihydroorotate dehydrogenase (DHODH), and GTP cyclohydrolase 1. These antioxidant enzymes result in generation of metabolites (such as coenzyme Q10 [CoQ10] and tetrahydrobiopterin [BH_4]) with radical-trapping antioxidant activity to inhibit lipid peroxidation (Figure 1). Most recently, a novel mechanism independent of both GPX4 and radical-trapping antioxidant activity has been discovered to suppress ferroptosis through Membrane Bound O-Acyltransferase Domain Containing 1 and 2 (MBOAT1/2)–mediated cellular PL remodeling.[16]

GPX4 Pathway in the Regulation of Ferroptosis

The intestinal mucosal epithelium is highly dependent on dietary cysteine whose deficiency can lead to impaired cell proliferation, defective barrier function, and cell death due to ferroptosis.[17] A rate limiting step in cellular GSH synthesis is the availability of cysteine, which plays an essential role in cellular redox homoeostasis. Cysteine is a key constituent of the GSH tripeptide that is required for GPX4 to inhibit lipid peroxidation and ferroptosis. Cysteine requires specialized transport systems for its import into the cell. System X_c^- is a dedicated cystine transporter that imports cystine in exchange for intracellular glutamate. System X_c^- is frequently up-regulated in cancer cells to counteract elevated levels of reactive oxygen species (ROS), making it an attractive target for anticancer treatment. Furthermore, system X_c^- contains a light chain subunit, SLC7A11, that is targeted by inhibitors such as sulfasalazine or the multikinase inhibitor sorafenib to induce ferroptosis.[18,19] *SLC7A11* expression can be repressed by the BRCA1-associated protein 1 (BAP1) that inhibits cystine uptake leading to elevated lipid peroxidation and ferroptosis.[20] Several noncoding RNAs[21–23] have also been shown to modulate expression of *SLC7A11* resulting in ferroptosis. Ionizing radiation (IR) can inhibit ferroptosis by inducing expression of both SLC7A11 and GPX4 as an adaptive response leading to radioresistance.[24] Interestingly, ferroptosis induced by erastin or RSL3 was shown to be enhanced by sodium butyrate (a short-chain fatty acid [SCFA]) by inducing lipid ROS production via down-regulation of the expression of SLC7A11, and GPX4.[25] The NRF2 transcription factor regulates GPX4[11] and SCL7A11[12] as well as many other lipid peroxidation- and ferroptosis-related genes such as metallothionein-1, an important regulator of lipid peroxidation[13] (Figure 1). Inhibition of NRF2 (using all-trans retinoic acid or brusatol) induces ferroptosis by mechanisms including sensitizing cells to erastin and sorafenib,[12] reducing metallothionein-1 expression,[26] and inducing redox imbalance and apoptosis in cancer cells.[27] There is also a synergistic effect of brusatol with lapatinib, an inhibitor of HER2/EGFR.[28]

Modulation of GPX4 activity regulates ferroptosis whereby inducers of ferroptosis, including statins, can indirectly down-regulate GPX4 expression or activity in cancer cells.[29] Conversely, up-regulation of GPX4 expression by activating transcription factors (TFAP2c and SP1) resulting from selenium supplementation can inhibit ferroptosis.[30] *MRP1,* a multidrug resistance gene,[31] functions to increase the cellular efflux of GSH,[32] and high levels of MRP1 expression (multidrug resistance phenotype) sensitize cancer cells to proferroptotic agents while conferring resistance to some proapoptotic anticancer drugs.[32] Accordingly, induction of ferroptosis can be exploited to target treatment-resistant cancer cells.

GPX4-Independent Pathways

The FSP1-CoQ10-NAD(P)H axis represents a parallel system to the GPX4-GSH axis for inhibition of lipid peroxidation and ferroptosis.[33,34] Of note, a combined administration of FSP1 and GPX4 inhibitors showed a more potent induction of ferroptosis compared with individual drugs.[35] The FSP1 can reduce vitamin K to its hydroquinone, which acts as an inhibitor of lipid peroxidation, thereby protecting cells from ferroptosis.[36] Other parallel systems that inhibit ferroptosis include DHODH,[37] and vitamin D3 has been shown to reduce cisplatin-induced intestinal injury by reversing down-regulation of both GPX4 and DHODH, explaining its antiferroptotic role.[38] The BH_4 biosynthesis pathway inhibits ferroptosis independent of cytosolic GPX4 or FSP1 (Figure 1). BH_4, a radical-trapping antioxidant cofactor, prevents membrane lipid peroxidation and its availability alters iron metabolism and mitochondrial function in T cells. Methotrexate inhibition of dihydrofolate reductase, an enzyme that regenerates BH_4, synergizes with GPX4 inhibition in the induction of ferroptosis.[39]

Iron Regulation and Iron-Induced Lipid Peroxidation

PL peroxidation can be initiated in cells through enzymatic and nonenzymatic processes. If PL hydroperoxide is formed and not neutralized promptly, it can undergo the iron-catalyzed Fenton reaction, resulting in generation of lipid hydroxyl and lipid peroxyl radicals. As the catalyst of the Fenton reaction, the cellular labile iron pool plays a crucial role in determining cellular susceptibility to ferroptosis. These lipid radicals propagate peroxidation to neighboring PL-containing polyunsaturated fatty acid (PUFA) chains, which can then react with oxygen to form lipid peroxyl radicals. This chain reaction amplifies PL peroxidation and ultimately leads to ferroptosis.

Cellular iron homeostasis is exquisitely regulated by iron regulatory protein-1 and iron regulatory protein-2 that control the level and activity of a series of protein factors involved in iron import, export, storage, and release, which coordinately maintain cellular iron homeostasis.[40,41] As expected, these protein factors can also modulate ferroptosis.[3,42] For example, a major mechanism for cellular iron uptake is mediated by transferrin and its receptor, TFRC, which mediates uptake of iron-bound transferrin that is required for ferroptosis induced by cysteine deprivation.[43] Plasma membrane expression of TFRC was shown to be significantly increased during ferroptosis and might serve as a specific ferroptosis marker.[44] Additionally, non transferrin-bound iron can be imported via encoded transmembrane proteins SLC39A8/ZIP8 and SLC39A14/ZIP14.

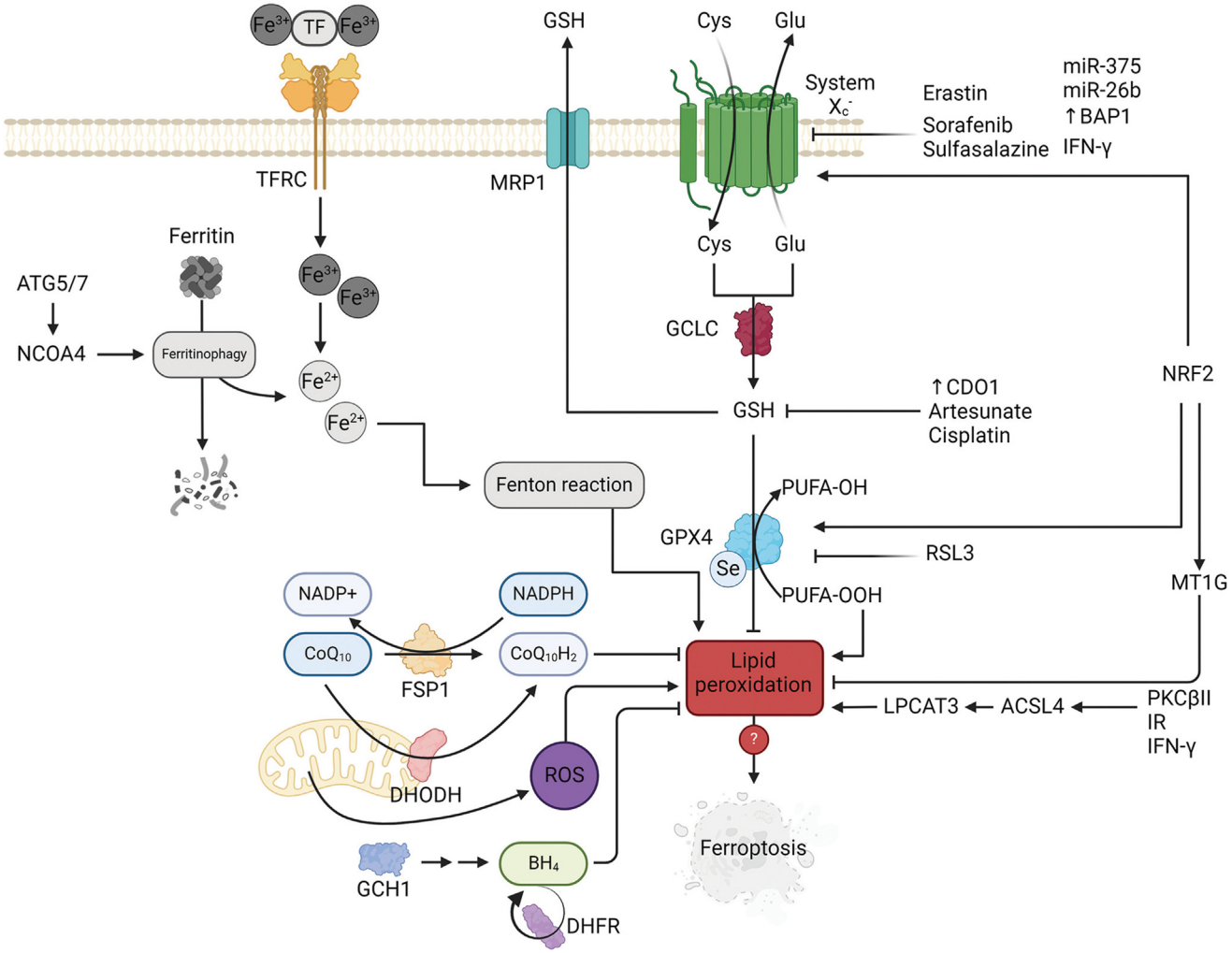

Figure 1. Pathways that can trigger ferroptotic cell death converge in the peroxidation of membrane lipids. End executors of ferroptosis remain to be elucidated. As a receptor in the plasma membrane, cystine-glutamine antiporter (system X_c^-) can be inhibited by erastin or glutamate to extrinsically induce ferroptosis, whereas inhibition of intracellular antioxidant enzymes (GPX4, FSP1, DHODH, GTP cyclohydroxylase-1 [GCH1], and DHFR) lead to intrinsically induced ferroptosis.[14] Depletion of GSH by MRP1 export,[32] up-regulated cys dioxygenase 1 (CDO1),[202] or treatment with cisplatin can also sensitize cells to ferroptosis.[203,204] The NRF2 pathway is involved in the expression of multiple proteins that can inhibit ferroptosis, including system X_c^-, GPX4, and MT1G. FSP1 catalyzes the regeneration of CoQ10[33,34] whose reduced form, ubiquinol, traps lipid peroxyl radicals that mediate lipid peroxidation and prevents ferroptosis induced by GPX4 depletion. GCH1 is the rate-limiting enzyme of the BH_4 biosynthetic pathway, which is another inhibitor of lipid peroxidation.[39] The lipid metabolism enzyme ACSL4, which preferentially acylates AA, and LPCAT3, which preferentially inserts acylated AA into membrane PLs, have critical roles in GPX4 inhibition-induced ferroptosis.[86,87] The first step in GSH synthesis is catalyzed by the glutamate-cysteine ligase catalytic subunit (GCLC) contributing to ferroptosis resistance.[205] An increase in the intracellular iron pool through ferritinophagy and TFRC also leads to enhanced lipid peroxidation.[43]

ZIP14-mediated iron uptake has been reported to promote ferroptotic liver injury.[45] An excessive amount of cellular iron can be exported by iron exporters such as ferroportin and prominin, and down-regulation of these exporters has been shown to promote ferroptosis.[46,47] Inside of cells, excess iron is mainly stored in a nontoxic form by the protein complex ferritin. As such, cytosolic ferritin and iron chaperone poly(rC)-binding protein 1 confer resistance to ferroptosis by limiting iron availability.[48,49] Conversely, iron can be released from ferritin through nuclear receptor coactivator 4–mediated ferritin-selective autophagy, known as ferritinophagy, which can increase labile iron content and increase the susceptibility of the cell to ferroptosis.[50,51]

The maintenance of mitochondrial iron homeostasis is also crucial in regulating ferroptosis. Mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) are essential mitochondrial iron importers involved in heme and Fe–S biogenesis. Mitoferrin 2 has been shown to promote ferroptosis likely through increased mitochondria iron content.[52] Mitochondrial Fe–S proteins are also involved in ferroptosis regulation,[53] and can prevent cancer cells from undergoing ferroptosis, probably through limiting mitochondrial iron level.[54,55] In contrast, heme oxygenase 1 (HO1), a mitochondrial enzyme that degrades heme to produce ferrous iron, leads to mitochondrial iron overload and sensitizes cancer cells to ferroptosis.[56,57] However, it

was also reported that mild up-regulation of HO1 protected cells from ferroptosis,[58] suggesting complex and a likely context-dependent role of HO1 in ferroptosis.

Metabolic Pathways

Ferroptosis can be considered as a natural outcome of cellular metabolism. PL-PUFA, a product of lipid metabolism and essential component of cell membranes, is the substrate for PL peroxidation, arguably the executing step of ferroptosis. On the other hand, ROS and free radicals, which initiate PL peroxidation and provide the initial trigger for ferroptosis, are inevitable products of cellular metabolism, particularly energy metabolism. Accordingly, various metabolic processes have been demonstrated to be important in ferroptosis of which examples are provided later in this article. Glucose starvation, which mimics an energy stress condition, inhibits lipid peroxidation and ferroptotic cell death of cancer cells. Inactivation of adenosine monophosphate-activated protein kinase (AMPK) serves to inhibit acetyl-coenzyme A (CoA) carboxylase and PUFA biosynthesis, and largely abolishes the protective effects of energy stress on ferroptosis.[59,60] Importantly, cancer cells with loss of function mutations of *LKB1*, a tumor suppressor and upstream AMPK activator, display high basal AMPK activity and show increased resistance to ferroptosis.[60] Conversely, glucose uptake mediated by SLC2A1 promotes glycolysis, pyruvate oxidation, tricarboxylic acid cycle (TCA), and fatty acid synthesis, which ultimately facilitates ferroptosis in cancer cells.[61]

Mammalian target of rapamycin complex 1 (mTORC1) is a nutrient sensor activated by amino acids, energy, and growth factors.[62] Oncogenic activation of the PI3K-AKT– mammalian target of rapamycin (mTOR) signaling axis was shown to suppress ferroptosis in cancer cells by increasing cellular monounsaturated fatty acid (MUFA) content. Conversely, inhibition of PI3K-AKT-mTOR signaling can sensitize cancer cells to ferroptosis induction.[63] Specifically, mTORC1 inhibition was shown to sensitize cancer cells to ferroptosis through decreasing GPX4 expression.[64] However, another study showed that adenosine triphosphate (ATP)-competitive mTOR inhibitors suppressed ferroptosis triggered by system X_c^- inhibition or direct cystine deprivation that may be due to up-regulated macropinocytosis of albumin that is an alternative source of cellular cysteine.[65,66] Therefore, the effect of mTORC1 on ferroptosis can be context dependent.

PL de novo synthesis and its remodeling process play significant roles in regulating ferroptosis sensitivity by determining the composition of the plasma membrane.[67] Various factors in these pathways have been shown to increase ferroptosis sensitivity including the uptake of essential PUFA by CD36,[68,69] the synthesis of long-chain PUFA by FADS1, fatty acid desaturase 2 (FADS2), and ELOVL5,[70,71] the activation of PUFA by ACSL4,[72–74] as well as the incorporation of PUFA-CoA into lyso-PL by LPCAT3.[72] Previous studies have demonstrated that exogenous MUFA[75] or de novo synthesized MUFA inhibits ferroptosis.[63] MBOAT1/2 selectively incorporates MUFA-CoA into lyso-phosphatidyl ethanolamine (PE), competitively inhibiting the synthesis of PE-PUFA. MBOAT1/2 suppresses ferroptosis in a GPX4-independent manner. Furthermore, the regulation of MBOAT1 and 2 is mediated by estrogen receptor and androgen receptor signaling, respectively, making them critical therapeutic targets in certain cancer types.[16]

Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) levels play a crucial role in sustaining redox homeostasis and cell survival.[76] Cellular NADPH abundance serves as a biomarker for predicting sensitivity to ferroptosis across different cancer cell lines.[77] NADPH can be produced through various metabolic pathways, including the pentose phosphate pathway and nicotinamide adenine dinucleotide (NAD) kinase–mediated phosphorylation of NAD. Suppressing NAD kinase reduces intracellular NADPH levels and increases cell sensitivity to ferroptosis.[77] Conversely, overexpression of MESH1, a cytosolic NADPH phosphatase that degrades NADPH into reduced nicotinamide adenine dinucleotide (NADH), depletes cellular NADPH and sensitizes cells to ferroptosis.[78]

Mitochondrial Metabolism

Mitochondria play a critical role in in cell homeostasis and cellular energy metabolism in eukaryotic cells. Although mitochondria provide intracellular ATP via oxidative phosphorylation, they also function as key mediators of various forms of RCD including apoptosis, pyroptosis, necroptosis, and ferroptosis. The involvement of mitochondria in ferroptosis was suggested based on the morphologic changes observed in ferroptotic cancer cells and that events associated with mitochondrial energy metabolism promote ferroptosis.[14,43,79] Accumulating evidence suggests that an impaired ferroptotic response is associated with changes in mitochondrial function. Mitochondrial ROS and the release of mitochondrial DAMPs contribute to inflammation in ulcerative colitis (UC).[80] Furthermore, Paneth cells are highly susceptible to mitochondrial dysfunction in Crohn's disease (CD).

To date, the role of mitochondria in regulating ferroptosis is poorly understood. Mitochondrial activity is crucial for cysteine-deprivation-induced (CDI) ferroptosis in contrast to RSL3-induced ferroptosis.[43,79] CDI ferroptosis fails to occur in the absence of glutamine because glutamine metabolism fuels the mitochondrial TCA cycle to enhance generation of mitochondria-derived ROS, which are necessary for lipid peroxidation during CDI ferroptosis. Metabolite intermediates of the TCA cycle, such as α-ketoglutarate, fumarate, succinate, and malate, can replace glutamine to induce CDI ferroptosis.[79] Inhibiting the TCA cycle or the electron transfer chain mitigates CDI ferroptosis.[79] Because mitochondria play a central role in ferroptosis initiation, cells have developed potential ferroptosis-suppressing mechanisms localized in the organelle, such as that mediated by DHODH and its product, reduced CoQ10.[37]

Mitochondria are the center of iron metabolism and energy production, leading to altered lipid peroxidation sensitivity. Mitochondria can modulate ferroptosis through mechanisms including the synthesis of Fe-S clusters. Additionally, mitochondrial Ca^{2+} plays a crucial role in triggering ferroptosis. Reducing Ca^{2+} influx can protect cells from ferroptosis induced by system X_c^- inhibitors such as erastin and sulfasalazine.[81] Recent studies have reported that

FUNDC2 regulates ferroptosis by interacting with the mitochondrial glutathione transporter SLC25A11 to negatively regulate mitochondrial GSH levels.[82] It has also been shown that FUNDC2 contributes to cardiomyopathy induced by the anthracycline doxorubicin through ferroptosis.[82] Beta-oxidation is generally believed to have a suppressive effect on ferroptosis by reducing the availability of unesterified PUFAs. The enzyme 2,4-dienoyl-CoA reductase 1 (DECR1), which is involved in PUFA beta-oxidation in the mitochondria, is overexpressed in prostate cancer. Knockout of *DECR1* induces endoplasmic reticulum (ER) stress and sensitizes castration-resistant prostate cancer cells to ferroptosis both in vitro and in vivo. Furthermore, inhibiting beta-oxidation enhances ferroptosis in cancer cells.[83,84]

As a major regulator of the antioxidant response, NRF2 plays a crucial role in regulating mitochondria respiration, controlling mitochondria-mediated ROS production, as well as the biosynthesis of glutathione and NADPH. It has been shown that NRF2 is a key determinant of the therapeutic response to ferroptosis-targeted therapies in hepatocellular carcinoma (HCC) cells.[12] Under oxidative stress, NRF2 is released from KEAP1-mediated ubiquitination. Stabilized NRF2 then accumulates in the nucleus, and transcriptionally activates a panel of genes that are involved in countering oxidative stress, including but not limited to: SLC7A11 (cystine transporter); G6PD and PGD (NADPH regeneration); ferritin and ferroportin (iron regulation); and FSP1 and DHFR (antioxidant generation), which serves as feedback regulation to maintain cellular redox homeostasis.[85]

Lipid Peroxidation

Lipid metabolic enzymes ACSL4 and LPCAT3 have fundamental roles in GPX4-induced ferroptosis (Figure 1).[86,87] ACSL4 preferentially activates arachidonic acid (AA) to AA-CoA, whereas LPCAT3 preferentially incorporates AA into membrane PLs.[86,87] Thus, inhibition of these enzymes prevents ferroptosis by reducing the pool of oxidation-sensitive fatty acids in cell membranes.[86] PKCβII catalyzes the phosphorylation and activation of ACSL4, thus amplifying lipid peroxidation that leads to ferroptosis.[88] Inhibition of PKCβII-ACSL4 blocks ferroptosis and limits ferroptosis-associated cancer immunotherapy.[88] IR induces ferroptosis in cancer cells through increased levels of ROS and up-regulation of ACSL4, leading to increased lipid peroxidation. Down-regulation of ACSL4 blocks IR-induced ferroptosis and enhances radioresistance.[24] In the context of immunotherapy, interferon gamma (IFN-γ) produced by $CD8^+$ T cells and AA up-regulate ACSL4 leading to lipid peroxidation and immunogenic tumor cell ferroptosis.[89] Whereas high levels of AA promote tumor ferroptosis, down-regulation of ACSL4 enhances tumor progression.[89] Importantly, low-dose AA administration was shown to enhance the therapeutic efficacy of PD-L1 blockade and improve anti-tumor T-cell responses through the IFN-γ signaling pathway.[89]

Ferroptosis in Gastrointestinal Disease

Common ferroptotic mechanisms in intestinal as well as other diseases include GPX4 inhibition, system X_c^- suppression, lipid peroxide accumulation, and iron overload. Key regulators, such as GPX4, SLC7A11, ACSL4, and p53, are also important for mediating ferroptosis-associated intestinal diseases. To date, the role of ferroptosis in gastrointestinal (GI) disease remains poorly characterized, and further research is needed to identify disease-specific ferroptotic mechanisms that may enable development of disease context-dependent therapeutic approaches. Studies have found correlations between ferroptosis and other forms of cell death in intestinal diseases that may share common pathways and key regulators, and may guide therapeutic interventions.

Iron overload is an important trigger of ferroptosis that can exacerbate intestinal inflammation. Regulation of iron absorption in enterocytes is dependent on the liver-secreted hormone hepcidin that forms a complex with ferroportin. When this complex enters iron-absorptive enterocytes, ferroportin is degraded resulting in iron accumulation with reduced delivery to plasma.[90] Excess intracellular iron that is unbound to ferritin (ie, labile cellular iron) has the potential to increase inflammation and lipid peroxidation resulting in cell death, including ferroptosis. Levels of hepcidin are influenced by gut microbiota, thus affecting iron uptake.[91] Iron overload can disrupt the gut microbiome to promote the growth of potentially pathogenic bacteria,[90] and resultant dysbiosis can lead to enhanced ferroptosis in intestinal epithelial cells. Although it has been reported that iron supplementation alters gut microbial homeostasis and exacerbates intestinal inflammation,[92] the specific mechanisms by which microbiota can affect ferroptosis await further study.

The intestinal epithelium is one of the fastest-renewing tissues in the human body. This rapid turnover requires significant metabolic activity and energy generation leading to increased ROS, iron metabolism,[93] and increased susceptibility to ferroptosis. Ferroptosis has been linked to disease generation through intestinal epithelial cell death mediated by ER stress.[92] Inflammatory responses triggered by ferroptosis can promote immunogenicity via activation of NF-κB by DAMPs.[94] Furthermore, lipid peroxidation products stimulate phagocytosis (via TLR2 by oxidized PL) and recruit macrophages by secreting chemokines.[95] Although these data suggest that inhibition of ferroptosis may be therapeutically beneficial, acquired resistance to ferroptosis with prevention of cell death has been shown to enhance neoplastic development and progression.[96] Although beyond the scope of this review, other forms of cell death distinct from ferroptosis occur in GI diseases, such as apoptosis, necrosis, necroptosis, and pyroptosis. Molecular cross-talk has been described between these forms of cell death and ferroptosis, and multiple forms of cell death can be observed in the context of the same disease.[97] The relative proportion each mechanism of cell death and its specific contribution to a disease entity awaits further research.[98–100]

Inflammatory Bowel Disease

Ferroptosis appears to play a role in inflammation-related conditions, such as inflammatory bowel disease (IBD), and has been implicated in both experimental colitis

and in human IBD. IBD is characterized by chronic and progressive inflammation of the GI mucosa, which has been shown to manifest features of ferroptosis including iron deposition, lipid peroxidation, and GPX4 inactivation.[92] Ferroptosis was shown to regulate colitis in the dextran sulphate sodium (DSS)-induced model of colitis, which showed increased lipid peroxidation.[92,101] Furthermore, excess iron can increase mucosal ROS production, which was shown to exacerbate intestinal inflammation in the DSS-induced colitis model.[92] Inhibition of ferroptosis was shown to reduce disease activity scores in DSS-induced colitis.[102]

ER stress signaling can mediate cell death signaling including ferroptosis, which was significantly increased in intestinal epithelial cells (IECs) from patients with UC and in mice with experimentally induced colitis.[92,102] Ferroptosis is recognized as an important contributor to IEC death in both humans and mice with UC.[14,92] In IEC-6 cells treated with H_2O_2, down-regulation of phosphorylated STAT3 was observed that could be reactivated by the ferroptosis inhibitor, Fer-1.[103] Phosphorylated NF-κBp65 can inhibit ER stress signaling by binding eukaryotic initiation factor 2α,[92] and deletion of *NF-κBp65* in IECs was shown to up-regulate ferroptosis and to exacerbate colitis. Iron overload has also been observed to induce colitis, enhance ferroptosis, and significantly alter the microbiota in mouse models resulting in modulation of the expression of several ferroptosis-related genes such as *SLC7A11* and *GPX4*.[104] GPX4 plays an important role by protecting against lipid peroxidation and ferroptotic cell death.[102] Of note, IECs derived from patients with CD were shown to exhibit impaired GPX4 activity and signs of lipid peroxidation.[105] PUFAs, and specifically AA, were shown to trigger a cytokine response in IECs that was restricted by GPX4.[105] Furthermore, a PUFA-enriched Western diet was shown to induce a granuloma-like neutrophilic enteritis in mice that lacked 1 allele of *Gpx4* in IECs, indicating that dietary PUFAs can serve as a trigger of GPX4-restricted mucosal inflammation with a phenotype of human CD. Together, these data indicate that ferroptosis may contribute to IBD via ER stress-mediated IEC cell death, and suggest that ferroptosis is a potential therapeutic target in IBD.[92]

The purported role of ferroptosis in IBD is believed to be linked to altered SCFA metabolism.[106] As previously stated, RSL3- and erastin-induced cell ferroptosis were shown to be enhanced by sodium butyrate. Specifically, sodium butyrate mediates the down-regulation of antiferroptosis proteins, SLC7A11 and GPX4, thus acting as a proferroptotic SCFA.[25] Down-regulation of these antiferroptotic proteins is mediated by the FFAR2-AKT-NRF2 and the FFAR2-mTORC1 axes.[25] Butyrate is a naturally occurring SCFA in the gut produced as a result of the microbial metabolism from dietary fiber. Butyrate is known to play a role in intestinal homeostasis and immune and epithelial barrier function. Butyrate is the primary energy source of IECs[107] and its mitochondrial oxidation accounts for >70% of the oxygen consumed in IECs.[106] In DSS-induced mice, iron chelation by deferasirox treatment strongly reversed the alterations caused by ferroptosis. Deferasirox was shown to significantly reduce DSS-induced UC in mice in association with a reduction in the level of proinflammatory cytokines (interleukin [IL] 1β, IL6, tumor necrosis factor [TNF]-α, and INF-γ).[108] Furthermore, deferasirox treatment reshaped the composition of intestinal microbiota, and metabolomics analysis indicated the SCFA production was enhanced in deferasirox-treated mice.

Targeting ferroptosis for therapeutic advantage has been demonstrated in animal models of colitis where inhibiting ferroptosis can attenuate intestinal injury in IBD. Suppression of ferroptosis was been shown to ameliorate DSS-induced colitis by blocking NRF2/HO-1 signaling.[101] Inhibition of ferroptosis by ferrostatin-1 was shown to alleviate colitis in the trinitrobenzenesulfonic acid–induced murine model.[102] In addition, a potent ROS scavenger (pH-sensitive molybdenum-based polyoxometalate nanocluster) was shown to attenuate ferroptosis and reduce inflammatory indicators in DSS-induced mouse models of UC.[109] Lipid peroxidation and elevated ferroptotic markers were also detected in the azoxymethane/DSS-induced mouse model of colitis-associated carcinogenesis (CAC). In this model, treatment with ferrostatin-1 was shown to increase the incidence of CAC induced by a high-fat diet, whereas induction of ferroptosis with RSL3 partially reversed this effect.[110] It should be noted that although ferrostatin-1 has been shown to inhibit ferroptosis,[14] its mechanism of action relies on preventing the accumulation of lipid peroxides (not a specific protein target), which hinders the potential to further improve the potency of the molecule.[111] Although ferrostatin has been used to inhibit ferroptosis, concerns exists regarding its efficacy as a ferroptosis inhibitor in vivo.

Ferrostatin-1 is unstable in plasma and has low blood-brain barrier permeability, which underscores the need for alternative inhibitors with improved pharmacokinetic characteristics.[111] In fact, there are already ferrostatin-1 analogs like liproxstatin-1 and UAMC-2418, which have better pharmacokinetic properties and outperform other inhibitors of ferroptosis in vivo.[112]

Ferroptosis in Liver Disease

Several forms of programmed cell death including apoptosis, necroptosis, and ferroptosis have been implicated in the pathogenesis of various liver diseases. Ferroptosis appears to be highly context dependent, which is further supported by its involvement in conditions affecting the liver. With respect to liver injury and non-neoplastic conditions, normal liver cells are susceptible to ferroptosis, whereas liver cancer cells display intrinsic or acquired resistance to ferroptosis. Accumulating evidence suggests that ferroptosis may serve as a promising target for the prevention and treatment of many forms of liver disease.

Ferroptosis has been linked to an expanding number of hepatic metabolic pathways[113] including the NADPH pathway,[114] the metabolism of fatty acids,[115] and amino acids such as cysteine.[116] Excessive iron regulates ferroptosis, and iron metabolism is primarily regulated by the liver, which produces transferrin and hepcidin to regulate dietary iron absorption.[117] Lower levels of these iron regulators have been found in patients with cirrhosis and liver

fibrosis compared with healthy controls.[45,118] In animal models, hepatocyte-specific knockout of the transferrin gene *Trf* coupled with a high-iron diet was shown to sensitize to ferroptosis-induced liver fibrosis that could be rescued with ferrostatin-1.[45] Recently, the antioxidant vitamin E has been proposed to limit ferroptosis in the liver and to decrease hepcidin levels leading to iron depletion.[119] However, it remains unknown as to whether abnormal levels of iron regulators, such as hepcidin, are a cause or a consequence of deregulated iron homoeostasis.[117]

Acute and Chronic Liver Injury

Acute liver injury frequently results from hepatotoxic agents like drugs, alcohol, ischemic injury, or viral infections.[120] Acetaminophen is a notable example, with studies indicating that ferroptosis inhibitors can moderately protect against acetaminophen-induced liver damage in mouse hepatocytes.[121] Patients with alcoholic liver disease (ALD) show ethanol-induced up-regulation of the TFRC in hepatocytes leading to iron overload.[122,123] Chronic alcohol consumption is also associated with down-regulation of hepcidin, resulting in increased iron transport and absorption in the intestine.[124] Iron overload leads to oxidative stress and hepatocyte ferroptosis, and both iron chelators and ferroptosis inhibitors can protect against ALD.[123] Moreover, ethanol has been shown to inhibit mitochondrial frataxin expression, a key player in iron homeostasis whose down-regulation leads to enhanced sensitivity to ethanol-induced ferroptosis, as do increased levels of ACSL4 and reduced system X_c^- and GPX4.[125] Restoring mitochondrial frataxin expression was shown to decrease sensitivity to ethanol-induced ferroptosis. Alcohol exposure also results in overexpression of fibronectin type III domain-containing protein 3B whose deletion in hepatocytes results in ethanol-induced steatosis through the inhibition of the AMPK pathway. Inhibition of AMPK down-regulates transferrin expression leading to iron overload and predisposes to increased lipid peroxidation and ferroptosis.[126] Other forms of cell death are also involved in alcohol-induced hepatotoxicity, including apoptosis and autophagy.[125]

Lipin-1, a lipid metabolic enzyme expressed in adipose tissue, is overexpressed in animal models of alcoholic steatohepatitis and was shown to enhance hepatic ferroptosis through the inhibition of adiponectin signaling.[127] Aberrant liver sirtuin 1 (SIRT1) is also implicated in the pathogenesis of ALD. Animal models of ALD with intestine-specific knock-out of *SIRT1* show reduced hepatic inflammation and liver injury, at least partially due to attenuated ferroptosis in the liver.[128] In mouse models of hepatocyte injury and acute-on-chronic liver failure (ACLF), which has been associated with ferroptosis, NRF2 activation ameliorates liver injury and protects against hepatotoxicity through inhibition of ferroptosis and inflammation.[129] Both ferroptosis and necroptosis have been found to contribute to ACLF through YAP signaling, and inhibition of the YAP pathway results in reduced liver fibrosis partially through inhibition of ferroptosis resulting from up-regulation of ACSL4 and down-regulation of SLC7A11.[130] Of note, YAP levels in plasma in patients with ACLF are increased compared with healthy controls.[130] Given the accumulated evidence linking ethanol consumption with ferroptosis in the liver, therapeutic strategies have the potential to reduce ethanol-related liver injury, although the precise molecular mechanisms await further study.

Viral Hepatitis

Infection with hepatitis B and hepatitis C are associated with development of liver fibrosis and in the setting of cirrhosis, can lead to HCC.[131] Exosomes derived from hepatocytes infected with hepatitis B virus (HBV) were shown to activate hepatic stellate cells and to promote fibrosis through the miR-222/TFRC axis.[132,133] In HCC cells, inhibition of miR-142-3p or overexpression of SCL3A2 were shown to inhibit ferroptosis and to reduce cell proliferation, migration, and invasion.[133] Other studies have revealed that arginine methyltransferase 9 expression is promoted by the HBV X protein, and targets the heat shock protein family A member 8 for arginine methylation resulting in up-regulation of CD44, which, in turn, inhibits ferroptosis and promotes HCC progression.[134,135] Low doses of selenium have been shown to inhibit the hepatotoxicity of HBV X protein through GPX4-mediated ferroptosis inhibition in cell lines and in mouse models.[136] The enzyme FADS2, which promotes lipid peroxidation, has emerged as a rate-limiting factor in hepatitis C virus replication, indicating a potential role of ferroptosis in hepatitis C infection. In hepatitis C–infected cells, overexpression of FADS2 was found to inhibit viral replication and sensitize cells to ferroptosis.[137]

Metabolic Dysfunction–Associated Steatotic Liver Disease

Metabolic dysfunction–associated steatotic liver disease (MASLD) is an increasingly prevalent condition that typically begins with steatosis and can progress to fibrosis and ultimately cirrhosis.[138] Many forms of cell death are involved in MASLD, including ferroptosis.[139] In patients with metabolic dysfunction–associated steatohepatitis (MASH), elevated end products of lipid peroxidation and iron overload are frequently observed, and iron overload correlates with the histologic severity of the liver disease.[139] Additionally, arachidonate 12-lipoxygenase, particularly in its interaction with acetyl-CoA carboxylase 1, has been found to promote the progression of MASH.[140] Murine models of MASH show that both a high-fat diet and iron overload induce lipophagy and ferritinophagy, respectively, and synergize in promoting ferroptosis that results in lobular inflammation and increased fibrosis.[141] Ferroptosis was shown to aggravate MASH progression whereas inhibition of ferroptosis decreased disease severity in a mouse model of MASLD induced by a high-fat diet.[142] Treatment with melatonin, a potent antioxidant, was shown to reduce hepatocyte ferroptosis by inhibition of ER stress through the MT2/cAMP/PKA/IRE1 pathway.[138] These studies suggest that inhibition of ferroptosis may be a potential therapeutic

approach to reduce or prevent liver damage in patients with MASLD.

Hepatic Fibrosis

A key step in development of liver fibrosis is the transdifferentiation of hepatic stellate cells (HSCs) into matrix-producing myofibroblasts.[143] In this context, regulators of ferroptosis in HSCs include RNA-binding proteins ELAV-like protein 1 (ELAVL1)[144] and zinc finger protein 36 (ZFP36).[145] On exposure to ferroptosis-inducing compounds, ELAVL1 protein expression was increased through inhibition of the ubiquitin-proteasome pathway. Although transfection with an *ELAVL1* plasmid induced ferroptosis, *ELAVL1* knockdown by small interfering RNA (siRNA) led to ferroptosis resistance. Up-regulated ELAVL1 expression also appeared to increase autophagosome formation and autophagic flux, which appeared to be the underlying mechanism for ELAVL1-enhanced ferroptosis.[144] In mice, treatment with sorafenib attenuated murine liver fibrosis by inducing HSC ferroptosis, which was impaired by HSC-specific knockdown of *ELAVL1*. Similarly and on exposure to ferroptosis-inducing compounds, a ubiquitin ligase (FBXW7/CDC4) decreased expression of the RNA binding protein ZFP36 whereas the *ZFP36* plasmid impaired *FBXW7* plasmid-induced ferroptosis in HSCs and inhibited induction of autophagy.[145] In mice, HSC-specific overexpression of *ZFP36* impaired erastin- or sorafenib-induced ferroptosis. Together, these results identify ELAVL1 and ZFP36 in the induction of autophagy-dependent ferroptosis and as potential targets for the treatment of liver fibrosis, although selective induction of ferroptosis in HSCs is needed with sparing of hepatocytes.

Hemochromatosis

Hereditary hemochromatosis is an iron-overload disease caused by mutations in genes involved in iron absorption.[146] Excessive iron is absorbed by the intestine and deposited in parenchymal cells leading to tissue damage and organ failure. Apart from genetic factors, environmental factors such as alcohol intake and blood loss can also influence iron accumulation. Excessive iron usually produces massive ROS through the Fenton reaction and subsequently leads to DNA damage and tissue injury. Treatment with iron was shown to induce ferroptosis in murine primary hepatocytes, and ferroptosis was also observed in mice fed a high-iron diet or in mouse models of hereditary hemochromatosis with severe iron overload, but not in mice with only mild iron overload.[114] Importantly, iron overload–induced liver damage was rescued by inhibition of ferroptosis by ferrostatin-1. Genes found to be significantly up-regulated in iron-treated cells and hemochromatosis include *SLC7A11*, a known ferroptosis-related gene, whose genetic deletion was insufficient to induce ferroptosis unless *SLC7A11*$^{-/-}$ mice were fed a high-iron diet. Data indicate that iron can up-regulate *SLC7A11* expression through the ROS–NRF2–antioxidant response element axis, which may be a potential compensatory mechanism to protect against iron overload–induced ferroptosis in hemochromatosis. In addition, SLC7A11 was shown to confer protection against ferroptosis during iron overload by uptaking cystine and reducing ROS production. In these models, iron-induced ferroptosis was not mediated by ER stress, the mitogen-activated protein kinase pathway, or autophagy.[114] These results suggest that ferroptosis may be a target for treating hemochromatosis-related tissue damage.

Ferroptosis and Cancer

Ferroptosis has multiple implications in tumor development. First, several cancer signaling pathways are involved in the regulation of ferroptotic cell death.[147] Moreover, cancer cells with dysregulated metabolism, a high accumulation of ROS, and specific mutations render some tumor cells more vulnerable to ferroptosis, and thus, create a therapeutic window for ferroptosis inducers as a therapeutic strategy.[147] Importantly, ferroptosis is triggered by several conventional cancer therapies such as chemotherapy, radiotherapy, immunotherapy, and targeted therapy.[147]

Cancer-Related Pathways in Ferroptosis

Several alterations in lipid metabolism have been described in cancer cells[148] including those that contribute to ferroptosis resistance such as activating mutations of *PI3K* or loss of PTEN function through downstream SREBP1/SCD1–mediated lipogenesis.[63] Furthermore, activation of the p62-Keap1-NRF2 pathway confers resistance to ferroptosis in HCC cells.[12] Genes regulated by NRF2, including *NQO1*, *HO1,* and *FTH1*, modify both iron metabolism and lipid peroxidation to confer ferroptosis resistance. Inhibition of the PI3K-AKT-mTOR signaling axis sensitizes cancer cells to ferroptosis induction with therapeutic potential through mTORC1 inhibition.[63] *SLC47A1* acts as a PL transporter[149] and can block ferroptosis through the inhibition of the ACSL4-SOART1 axis that leads to lipid peroxidation. Targeting SLC47A1 sensitizes tumor cells to ferroptosis induction and may be a strategy for tumor suppression and overcoming drug resistance.[149] Studies indicate that SLC27A5/FATP5, an enzyme involved in the metabolism of fatty acids and bile acids, is down-regulated in HCC cells that are resistant to the multikinase inhibitor, sorafenib. SLC27A5 deficiency facilitates this resistance by suppressing ferroptosis. Mechanistically, loss of SLC27A5 enhances glutathione reductase expression in a NRF2-dependent manner, and renders HCC cells insensitive to sorafenib-induced ferroptosis in vitro and in vivo.[150]

TP53 acts as a metabolic regulator and inhibits cysteine uptake, which sensitizes cells to ferroptosis through transcriptional repression of *SLC7A11*.[151] SAT1 expression, which is transcriptionally regulated by *p53*, can trigger lipid peroxidation and sensitize cells to ROS-induced ferroptosis.[152] Moreover, SAT1 increases expression of arachidonate 15-lipoxygenase (ALOX15), which catalyzes peroxidation of AA, and is a key metabolic regulator in turning oxidative stress into lipid peroxidation.[153] SAT1 expression is down-regulated in human tumors possibly contributing to resistance to ferroptosis, and inhibition of ALOX15 abrogates

SAT1-induced ferroptosis.[152] On the other hand, p53 has also been shown to promote ferroptosis resistance such as via transcriptionally up-regulating p21 and, thus, decreasing cellular metabolism[154] or via nontranscriptionally blocking the activity of dipeptidyl peptidase-4 (DPP4), which is involved in lipid peroxidation through unknown mechanisms.[155]

Intercellular interactions mediated by E-cadherin regulate ferroptosis through the intracellular NF2-Hippo signaling pathway. Inhibition of this axis leads to YAP signaling up-regulation of ferroptosis modulators, such as ACSL4 and TFRC.[156] As alterations in the cadherin-NF2-Hippo-YAP signaling axis are common in cancer and can sensitize to ferroptosis, this signaling axis may be therapeutically targeted.[156] Furthermore, because of the role of YAP signaling in epithelial-mesenchymal transition (EMT), the function of YAP to promote ferroptosis provides a mechanism explaining why therapy-resistant mesenchymal tumor cells are often more susceptible to ferroptosis induction and are addicted to GPX4 activity.[29] Moreover, dependency on GPX4 is correlated with high level expression of ZEB1 in therapy-resistant tumor cells,[29] which links mesenchymal gene expression with lipid metabolism via direct regulation of the transcription of peroxisome proliferator activated receptor gamma and EMT-associated remodeling of the plasma membrane.[29] Selective sensitivity of ZEB1-overexpressing cells to lipid-peroxide activity and ferroptosis can be achieved by targeted inhibition of GPX4 or GSH for therapeutic advantage.[29] In addition, the *RAS* family of proto-oncogenes are among the more frequently mutated genes in cancers. Transcription of *SLC7A11* is controlled by oncogenic RAS signaling through ETS-1 in a regulatory network that allows *RAS*-driven tumors to evade ferroptosis.[157] This creates an opportunity for targeting cystine uptake and GSH biosynthesis through pharmacologic targeting of system X_c^- in *RAS*-driven tumors, which has been shown to impair tumor growth in vivo.[157]

Ferroptosis and Colorectal Cancer

Colorectal cancer (CRC) is the third most common human cancer[158] and ranks fourth as a cause of cancer-related death worldwide.[158] Patients with metastatic disease are generally incurable, and drug resistance underlies treatment failure, which underscores the need for new therapies to improve patient survival.

Regulation of Ferroptosis in CRC

Tumor cell ferroptosis is a complex and context-specific process. In CRC cells with intrinsic resistance to ferroptosis induction, simultaneous inhibition of GPX4-GSH and FSP1-CoQ10 axes was insufficient to restore sensitivity, but also required inhibitors of glycolysis or the Warburg effect.[159] In contrast to *TP53* transcription-dependent induction of ferroptosis reported in other tumor cell types, TP53 can block erastin-induced ferroptosis in CRC cells through the inhibition of DPP4 activity in a transcription-independent manner by promoting its localization to the nucleus where it is inactive.[155] Down-regulation of TP53 enables DPP4-dependent lipid peroxidation that ultimately leads to ferroptosis.[155] CRC cells can be protected from ferroptosis in a TP53-dependent manner by degradation of the RNA binding protein heterogeneous nuclear ribonucleoprotein C mediated by the E3 ubiquitin ligase, cullin-9.[160] Downstream alternative splicing targets of heterogeneous nuclear ribonucleoprotein C include *TP53* and *SLC47A1* messenger RNA.[160] The TP53-induced glycolysis and apoptosis regulator is overexpressed in CRC, and its activity increases available NADPH to convert oxidized glutathione to GSH and to reduce ROS levels.[161] Down-regulation of TP53-induced glycolysis and apoptosis regulator sensitizes CRC cells to erastin-induced ferroptosis via increased intracellular ROS, activation of AMPK, and down-regulation of SCD1.[161] Hypoxia-inducible factor 2α is involved in CRC progression, and its activation triggers proinflammatory responses in epithelial cells,[162] and also renders CRC cells vulnerable to ferroptosis through up-regulation of lipid and iron regulatory genes.[162]

Butyrate not only has important effects in the induction of ferroptosis but also in preventing the development of CRC through the inhibition of the activation of mTOR1.[25] In CRC cells, butyrate induces ferroptosis through both the increased expression of ACSL4, which increases the susceptibility of cells to lipid peroxidation,[163] and reduction of system X_c^- expression, which limits cysteine availability.[164] It should be noted that in CRC, system X_c^- expression is higher compared with tumor-adjacent tissue.[164] Butyrate has been shown to enhance the proferroptotic effects of the cytotoxic chemotherapy drug oxaliplatin in CRC cells in vitro and in vivo.[164] The combined intake of highly fermentable dietary fiber to enhance butyrate production and of omega-3 PUFAs has been proposed to reduce the risk of CRC. These treatments can promote ferroptosis through GPX4 and mitochondrial metabolism pathways.[107] The "butyrate paradox," where butyrate enhances proliferation of normal cell but not of cancer cells, also shows the context-dependent nature of its metabolism.[25]

Several genes and RNA molecules with altered expression in CRC have been associated with ferroptosis regulation and tumor development.[158] Furthermore, many ferroptosis-related gene signatures, deposited in public databases, have been proposed to prognosticate patients with CRC,[158] however, limitations exist and there is the need for validation. These signatures have the potential to identify important regulatory pathways of ferroptosis in CRC that may lead to development of new therapeutic strategies.

Therapeutic Strategies Involving Ferroptosis in CRC

Induction of ferroptosis is a potential approach for the treatment of CRC, and several drugs have been associated with this form of RCD.[158] A subset of cancer cells may avoid therapy-induced cell death by switching to a slow, yet reversible proliferation state that is drug tolerant. Drug-tolerant persistent CRC models show increased GPX4 expression making them vulnerable to GPX4 inhibitors,[165] but more potent and bioavailable compounds to achieve GPX4 inhibition are desirable.[165] Overexpression of lipocalin

2 (LCN2), involved in the regulation of iron homeostasis, in CRC cells promotes chemoresistance to 5-fluorouracil through the inhibition of ferroptosis.[166] Conversely, inhibition of LCN2 using monoclonal antibodies was shown to sensitize cells to chemotherapy and to inhibit tumor growth.[166] However, LCN2 was also shown to inhibit the NF-κB/SNAIL pathway and to prevent EMT and metastasis of CRC. Conflicting data exist in that silencing of LCN2 was shown to promote tumor growth[167] and, thus, the role of LCN2 in CRC progression remains controversial. It is established that *KRAS*-mutated CRC cell lines are resistant to therapy with EGFR inhibitors, including cetuximab.[168] Inhibition of the NRF2/HO-1 axis by cetuximab has been shown to promote ferroptosis induced by RSL3 in *KRAS*-mutated CRC cells.[168]

In the context of clinical application for cancer therapy, ferroptosis can be targeted by exploiting cancer cell regulatory alterations that result in enhanced metabolism to sustain increased requirement for energy and metabolite synthesis. These changes can make cancer cells more susceptible to ferroptosis (for example, by being more dependent on GPX4 activity or other ferroptosis surveillance mechanism) creating therapeutic windows for ferroptosis inducers in specific cancers. Cancer cells that have acquired resistance to ferroptosis can be resensitized by targeting the specific mechanisms that protect them. Furthermore, ferroptosis inducers can sensitize cancer cells to conventional anticancer therapies or overcome acquired resistance to thereby improve efficacy.[147]

The Roles of Ferroptosis in the TME and Immunotherapy

The pros of ferroptosis in immunotherapy. The release of DAMPs is one of the major features of immunogenic cell death that activates the immune system and synergizes with immune checkpoint blockade (ICB). HMGB1 is a DAMP that is released by ferroptotic cells in an autophagy-dependent manner. Of note, the ATG5/ATG7 autophagic axis is necessary for the release of acetylated HMGB1 during ferroptosis.[169] Other immunogenic DAMPs, including ATP, CRT, and DCN, were also confirmed to be released by ferroptotic cells.[170]

Moreover, recent studies suggested that CD8+ T cells can kill cancer cells via ferroptosis. IFN-γ secreted by activated CD8+ T cells down-regulates the expression of SLC7A11 in cancer cells by JAK-STAT1 pathway and sensitizes cancer cells to ferroptosis.[171] IFN-γ in combination with AA can further promote ferroptosis in cancer cells. IFN-γ up-regulates ACSL4 expression through the STAT1/IRF1 signaling pathway in cancer cells, promotes the incorporation of AA into PLs, and sensitizes cancer cells to ferroptosis. Combining AA and ICBs can synergistically inhibit tumor growth through IFN-γ.[89] These studies have indicated a synergistic effect between ferroptosis induction and ICBs, representing a potential treatment strategy.

The cons of ferroptosis in immunotherapy. Chronic inflammation can lead to tumor initiation, growth, and progression by providing a tumor-supportive microenvironment.[172] Tumor cells release proinflammatory molecules that promote immune evasion, angiogenesis, and growth of cancer stem cells.[8,172] Several immunosuppressive cell types have been identified in the TME, including myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAMs), and regulatory T cells (Tregs),[173] which may participate in ferroptosis-related immunosuppression. Although induction of ferroptosis to target tumors resistant to conventional therapies is promising, the resulting release of proinflammatory signals can have a negative impact on ferroptosis-based therapies for cancer. Cancer cell immune evasion involves mechanisms including macrophage polarization, impaired immune cell cytotoxicity, and up-regulation of immunosuppressive cells.[8,172] Importantly, ferroptosis plays a role in all of these mechanisms in the TME (Figure 2).

The release of HMGB1 has implications for immune responses and cancer, including the proinflammatory TME.[8,174] The binding of HMGB1 to toll-like receptor 4 and advanced glycosylation end-product specific receptor mediates immune responses such as the release of the cytokine TNF.[169] Mutated $KRAS^{G12D}$ acts as a DAMP during ferroptosis whose release after autophagy-dependent ferroptosis activates advanced glycosylation end-product specific receptor and leads to macrophage polarization through STAT3-dependent fatty acid oxidation.[175] Alternatively activated or M2 macrophages is associated with tumor progression due to their angiogenic[176] and immunosuppressive[177] activities. In mutated $KRAS^{G12D}$ pancreatic tumors, tumorigenesis is promoted by depletion of GPX4 or high-iron diets leading to ferroptosis.[178] The release of 8-hydroxyguanosine during ferroptosis activates a DNA-sensing pathway that regulates inflammation and immune responses, causing macrophage migration and activation.[178] Polarization of macrophages is also promoted by down-regulation of Acyl CoA dehydrogenases enzyme, which can also enhance CRC tumor growth.[179] Interestingly, M2 macrophages are more susceptible to ferroptotic cell death than M1 macrophages.[180] Up-regulation of NOS2 in M1 macrophages protects them from lipid peroxidation, whereas down-regulation of NOS2 is promoted in proinflammatory conditions including those of the TME.[180]

Treg cells are protected from ferroptosis by GPX4, sustaining their activation and antitumor immune activity in the TME.[181] Treg-specific deletion of GPX4 limits tumor growth and enhances antitumor immune responses, such as production of proinflammatory cytokines including IL1β.[181] MDSCs, which inhibit T-cell–mediated antitumor immunity, are protected from ferroptosis through N-acylsphingosine amidohydrolase overexpression. N-acylsphingosine amidohydrolase targets and suppresses the p53/Hmox1 axis reducing lipid peroxidation and preventing ferroptosis.[182] One of the mechanisms of MDSCs for blocking T-cell activation is the depletion of cysteine. T cells lack both cystathionase (that converts methionine to cysteine) and a complete system X_c^- (to import cystine), whereas MDSCs do express a functional system X_c^- but not the ASC neutral amino acid transporter that exports cysteine.[183] MDSCs block T-cell activation by sequestering cystine and limiting its availability, which leads to T-cell ferroptotic vulnerability

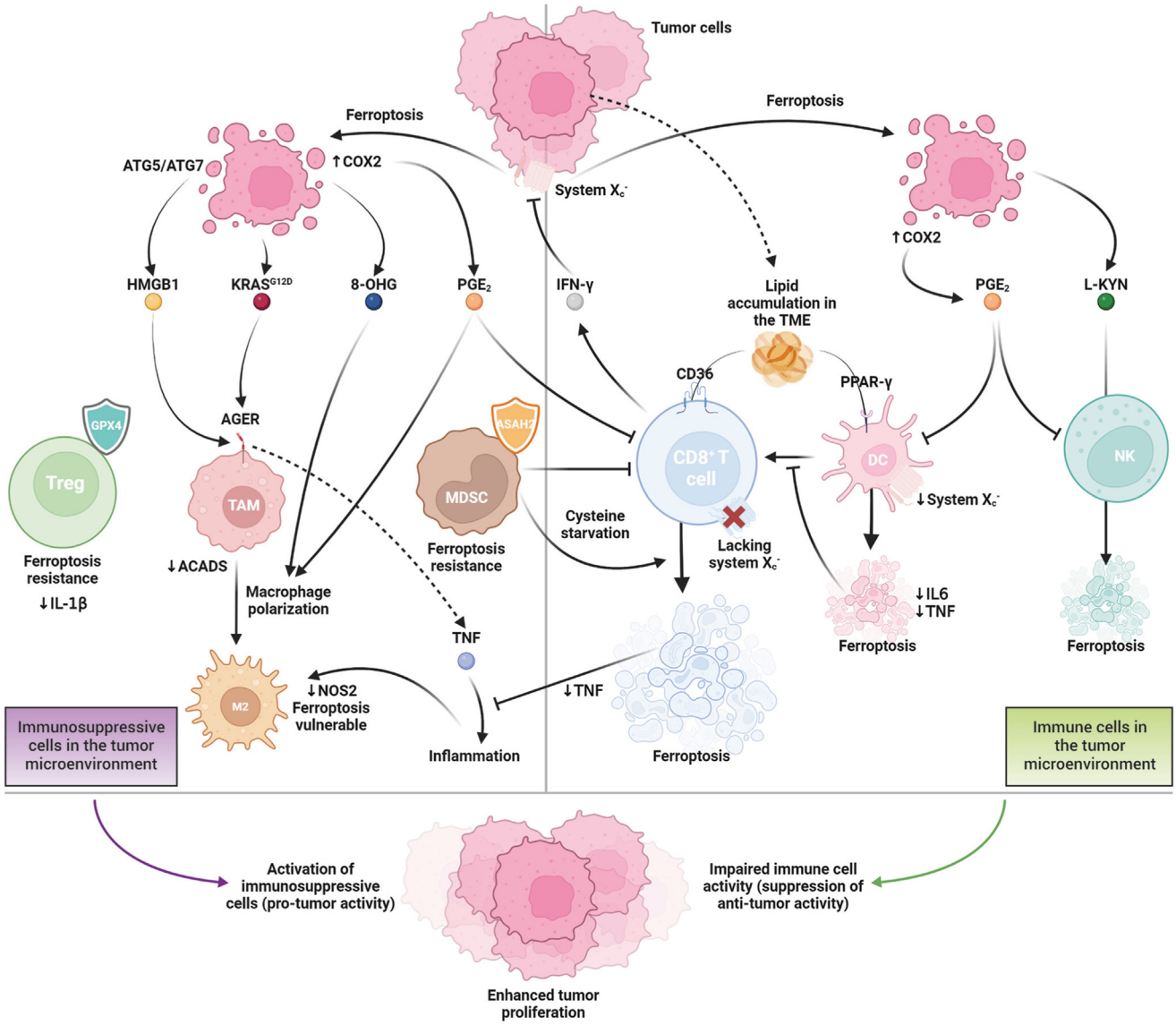

Figure 2. Involvement of ferroptosis in the TME and modulation of immune responses. Tumor cell ferroptosis promotes the activation of immunosuppressive cells and impairs the activity of antitumor immune cells. Immunosuppressive Tregs and MDSCs are protected from ferroptosis through up-regulation of GPX4 and N-acylsphingosine amidohydrolase, respectively. Down-regulation of Acyl CoA dehydrogenases (ACADS), and exposure to HMGB1, $KRAS^{G12D}$, PGE_2, and 8-OHG resulting from tumor cell ferroptosis in the TME leads to macrophage polarization with an M2 phenotype. TAMs' release of proinflammatory TNF is dependent on HMGB1 interaction with the advanced glycosylation end-product specific receptor (AGER) receptor. The inflammatory environment reduces NOS2 expression in M2 macrophages and makes them more vulnerable to ferroptosis. Active $CD8^+$ T cells release IFN-γ that impairs tumor cancer cell expression of system X_c^- that results in ferroptotic cell death. Ferroptotic cancer cells increase the expression of cyclooxygenase-2 that elaborates PGE_2, which serves to inhibit immune cell ($CD8^+$ T cells, DCs, and natural killers) activities. $CD8^+$ T cells are led to ferroptosis through both cysteine depletion as a consequence of their inactive system X_c^- and accumulation inside MDSCs and by increased lipids in the TME that are uptaken through the CD36 membrane receptor. In DC cells, down-regulation of system X_c^- and increased lipids in the TME (activating the peroxisome proliferator activated receptor gamma receptor pathway) enhance ferroptosis and impair their activation of $CD8^+$ T cells and their release of proinflammatory IL6 and TNF. L-KYN release from ferroptotic cancer cells in the TME leads to ferroptosis of natural killer cells.

in the setting of a TME that is enriched in MDSCs. Mouse models with T cells lacking GPX4 have reduced peripheral $CD8^+$ T cells and impaired response to infection, whereas both $CD4^+$ and $CD8^+$ T cells lacking GPX4 underwent ferroptotic cell death in vitro.[184] Also, $CD8^+$ T cells are more sensitive to ferroptosis induction using inhibitors of GPX4 activity than are cancer cell lines.[185] Membrane glycoprotein CD36 mediates ferroptosis of $CD8^+$ T cells through its fatty acid uptake activity, impairing $CD8^+$ T-cell antitumor activity.[69] Mechanistically, the uptake of oxidized low-density lipoproteins by CD36 induces p38 phosphorylation, and this activation limits secretion of TNF and IFN-γ

(effectors of $CD8^+$ T-cell immune activity) while also inducing lipid peroxidation and ferroptosis.[186] Knockout of *ACSL4* protects these cells from ferroptosis but impairs their antitumor activity, whereas overexpression of GPX4 or FSP1 also protects from ferroptosis while retaining their functionality.[185] In fact, overexpression of GPX4 not only protects $CD8^+$ T cells from ferroptosis, but also enhances their immune activity.[186] IFN-γ released from immunotherapy-activated $CD8^+$ T cells down-regulates the expression of system X_c^- in tumor cells, promoting lipid peroxidation and ferroptosis[171] while also sensitizing to radiotherapy.[187] Because IFN-γ induces expression of PD-L1 and promotes tumor progression,[188] the addition of immune checkpoint blockade should be considered as a potential combination therapy. Apolipoprotein L3 promotes ferroptosis of CRC cells and inhibits tumor growth through L-lactate dehydrogenase A ubiquitination and degradation in the proteasome.[189] This pathway enhances the antitumor activity of $CD8^+$ T cells increasing the levels of IFN-γ and reducing lactic acid concentration.[189] The combination of overexpression of apolipoprotein L3, ferroptosis induction with RSL3, and inhibition of PD-L1 has a synergistic effect in CRC models.[189]

Other immune cell types have impaired cytotoxic activity in the TME. In this regard, the activity of natural killer cells is down-regulated in the TME,[190] whereas activation of the NRF2 signaling pathway restores their antitumor activity.[190] Dendritic cells (DCs) are antigen-presenting cells that are important for the activation of cytotoxic T lymphocytes.[191] Moreover, DC functions are impaired by lipid peroxidation byproducts that cause ER stress.[192] DC induction of ferroptosis can be achieved with the RSL3 inhibitor of GPX4, but not with the SLC7A11 inhibitor erastin (due to the already low expression of SLC7A11 in DCs).[193] Mechanistically, RSL3-induced ferroptosis in DCs is dependent on peroxisome proliferator activated receptor gamma, acting as a key transcription factor for lipid metabolism.[193] DCs undergoing ferroptosis lose their immune functions such as production of proinflammatory cytokines (TNF and IL6) and activation of $CD8^+$ T cells.[193] Moreover, engulfment of ferroptotic dying cancer cells by DCs not only impedes antigen cross-presentation in DCs, but also impedes the immunogenicity of chemotherapy-induced apoptotic cells, resulting in decreased proliferative capacity of cytotoxic T lymphocytes.[194]

Polymorphonuclear (PMN)–MDSCs in the TME are highly sensitive to ferroptosis. Interestingly, spontaneous ferroptosis of PMN-MDSCs does not enhance antitumor immunity by reducing the number of PMN-MDSCs. Instead, it confers immunosuppressive activity on PMN-MDSCs. Ferroptotic PMN-MDSCs release prostaglandin E2 (PGE2) and oxidized phospholipids, influencing the activity of CD8+ T cells and TAMs and rendering a more immunosuppressive TME. Inhibition of ferroptosis in immunocompetent mice abrogates the immunosuppressive activity of PMN-MDSCs, particularly when combined with ICBs, and reduces tumor progression. This indicates that PMN-MDSC ferroptosis may be a targetable immunosuppressive mechanism for cancer immunotherapy.[195] Overexpression of cyclooxygenase-2 in CRC and other cancer cell types[196] has been reported in cancer cells undergoing ferroptosis.[15] COX-2 enzymatic activity is the rate-limiting step in PGE2 synthesis whose membrane receptors can inhibit antitumor immune responses.[197,198] The COX-2-PGE2-EP axis suppresses activation of myeloid cells[197] and the activity of DCs, natural killer cells, and T cells, while promoting polarization of TAMs to an M2 phenotype,[198] thereby contributing to tumor immune escape. Future studies are needed to clarify the immunomodulatory effects of ferroptosis, including its association with diseases outcomes. In addition, further study of ferroptosis-related signal transduction by different immune cell subpopulations is awaited.

Targeting Tumor Ferroptosis to Improve the Efficacy of Immunotherapy

Due to the heterogeneity and high complexity of TME, ferroptosis-inducing agents can cause either immune active or immunosuppressive function in a TME-dependent manner. Therefore, it demands the development of ferroptosis-inducing agents that selectively induce tumor ferroptosis and improve the efficacy of immunotherapy. SLC7A11 had been shown to be dispensable for proliferation of T cells in vivo and for their antitumor immune responses. Consequently, tumor cell SLC7A11 loss acts synergistically with the immunotherapeutic agent anti-CTLA4, laying the foundation for using specific SLC7A11 inhibitors to expand the efficacy of existing anticancer immunotherapeutics.[199] Recently, a small molecule compound, N6F11, had been shown to selectively trigger degradation of GPX4 in tumor cells but not immune cells. N6F11 caused ferroptotic cancer cell death, initiated HMGB1-dependent antitumor immunity, and sensitized immune checkpoint blockade in advanced cancer models.[200] Another study showed that deubiquitinase inhibitor PR-619 degraded GPX4 and increased the efficacy of anti-PD1 in a colon cancer model.[201] These findings may establish a safe and efficient strategy to boost ferroptosis-driven antitumor immunity by targeting GPX4 selectively in tumor cells.

Conclusions

Ferroptosis is a form of metabolically RCD. Data continue to emerge regarding ferroptotic regulatory pathways and the relationship between ferroptosis and pathophysiology of diseases including those affecting the GI tract. Iron overload, ROS accumulation, lipid peroxidation, and impaired antioxidant systems are critical steps in pathways regulating ferroptosis that are involved in GI diseases. Although studies have used the accumulation of lipid peroxidation as an indicator of ferroptosis, this process can also be part of other types of RCD including apoptosis, necroptosis, or pyroptosis. Although much progress has been made in understanding pathologic roles of ferroptosis, critical questions remain to enable development of ferroptosis-targeted therapies. Ferroptosis appears to have dual roles whereby inhibition of ferroptosis can alleviate intestinal damage, whereas induction of ferroptosis has

been shown to inhibit CRC cell migration and proliferation. There is an unmet need to identify specific biomarkers of ferroptosis and further research is required to detect ferroptotic mechanisms that are specific for individual disease entities. Therapeutic opportunities related to ferroptosis hold promise for therapy of multiple disease states. Cellular susceptibility to ferroptosis is dependent on the in vivo microenvironment, and vulnerability to ferroptosis among cancer cells, immune cells, and within the TME awaits further study. It remains to be determined if we can activate ferroptosis specifically in cancer cells without affecting healthy cells. Studies suggest that ferroptosis plays an important role in tumor suppression as well as resistance to cancer therapy, which can provide novel therapeutic opportunities. In conclusion, ferroptosis is a mechanistically and morphologically distinct form of RCD that has provided insights into pathophysiology of multiple disease entities. Elucidating the regulatory pathways related to ferroptosis can provide new opportunities for understanding pathophysiology of disease including cancer and identifying novel targets and strategies for exploitation of ferroptosis for therapeutic advantage.

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Conflicts of interest
The authors disclose the following: D. Liang is an inventor on a patent related to autophagy. X. Jiang is an inventor on patents related to autophagy and cell death; and holds equity of and consults for Exarta Therapeutics and Lime Therapeutics. J.J. Escuder-Rodríguez is affiliated with Instituto de Investigación Biomédica de A Coruña (A Coruña, Spain). The remaining author discloses no conflicts.

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