Ferroptosis Resistance in Cancer: An Emerging Crisis of New Hope

Daiyun Xu¹, Yonghui Lü¹, Yongxiao Li¹, Shengbin Li¹, Zhe Wang²,* and Junqing Wang¹,*

Introduction

The unregulated proliferation, metastasis, and chemoresistance in cancer progression are pressing challenges in cancer treatment. This severe outcome primarily arises from intrinsic insensitivity or adaptive evasion of apoptosis [1, 2]. Ferroptosis is a recently discovered new mode of regulated cell death characterized by an iron-dependent form of phospholipid peroxidation induced by reactive oxygen species (ROS) [3, 4]. With the recognition that ferroptosis can be exploited to eradicate chemoresistance malignancies in an apoptosis-independent manner, several druggable targets for inducing ferroptosis have been discovered. These include: 1) direct inhibition of glutathione (GSH) production via class I ferroptosis inducers (FINs) (e.g., erastin [5], 5-octyl d-glutamate [6], sorafenib [7], sulfasalazine [8], acetaminophen [9], buthionine sulfoximine (BSO) [4]; 2) direct GPX4 inactivation using class II FINs (e.g., altretamine [10], ML-162 [4, 10, 11], ML-210 [4, 10, 11], RSL3 [3, 4], withaferin A [12], FIN56 [13, 14]), and 3) other non-canonical approaches such as iron-based nanoparticles and iron oxidizers (e.g., C’ dots [15], artemisinin [16], artesunate [17], FINO₂ [14–17]), as well as some specific polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) may provide alternative strategies to induce ferroptosis in cancers.

Ferroptosis execution can be categorized into canonical and noncanonical pathways [18], which refers to the inactivation of the central antioxidant system (canonical) and Fe²⁺ catalyzed ROS overload, respectively. However, distinct ferroptosis sensitivity patterns have been observed among various cancer cell types due to irregular iron metabolism [19], anti-oxidative defenses [20–22], and altered lipid metabolism [23]. Recently, the underlying mechanisms associated with ferroptosis susceptibility have been progressively revealed, especially in light of how cancer cells evolve adaptations...
to ferroptosis-resistant states in response to ferroptotic stress upon pharmacological interventions.

In accordance with the current molecular understanding of ferroptosis susceptibility for cancer treatment, this opinion article briefly summarizes the current understanding of ferroptosis as a means of chemoresistant cancer treatment. In particular, we highlight the role of iron and lipid metabolism in anti-oxidative defense. We also discuss emerging mechanisms regarding ferroptosis resistance to provide further insight for proposing new opportunities and potential pitfalls.

**Programed cell death: the significance of ferroptosis**

The phenomenon of ferroptosis in cancer cells originated in 2003 with the recognition that a specific quinazolinone compound erastin, promotes a novel iron-dependent cell death with the mutant RAS oncogene [24]. Later, this finding was further confirmed by identifying the Ras-selective lethal (RSL) small molecule RSL-3 and RSL-5 in 2008 [25]. Unlike other classic cell death modalities such as apoptosis, necrosis, and autophagy, ferroptosis lacks nuclear morphological changes, cellular blebbing, and biochemical features involving caspase activation, LC3 conversion (LC3-I to LC3-II), and ATP depletion [3]. In contrast, this new mode of regulated cell death is characterized by morphological shrinkage and increased density of the mitochondrial membrane. At the biochemical level, ferroptosis mainly occurs with malfunctions of the system XC−/glutathione (GSH)/glutathione peroxidase 4 (GPX4) pathway and leads to uncontrolled peroxidation of phospholipids containing PFUs (PUFA-PLs) within the cell membranes [3, 4, 26]. The mechanistic relationship between lipid peroxidation and cell ferroptosis is still largely elusive. Recent studies have suggested that ROS free radicals could oxidize PUFA-PLs in cell membranes, and this consequence of the lipid compositional changes leads to membrane destruction, to membrane pores opening, and subsequent cell swelling followed by membrane rupture [27, 28]. Besides, this ferroptosis-associated cell rupture effect can spontaneously spread to neighboring cell populations through lipid peroxide in an iron-dependent manner [27]. Additionally, oxidized PUFAs and toxic by-products such as aldehydes and Michael acceptors may also disrupt the function and activity carried out by intracellular proteins and activate other fatal events downstream [29–31].

**The role of iron metabolism in lipid peroxidation**

Ferroptosis is an iron- and PUFA-PL peroxidation-dependent membrane dysfunction that results in regulated cell death [29]. The regulation of ferroptosis involves multiple genes and pathways associating with cellular homeostasis in iron metabolism, lipid synthesis, and redox status [18, 32]. PUFA-PLs were critical initiators of ferroptosis due to their high oxidation susceptibility, and this oxidative lipid damage can be driven by: 1) a free-radical chain reaction (autoxidation) and 2) an iron-dependent enzymatic oxygenation [33–35]. The initial step of the free-radical chain reaction is triggered by an ROS, including hydroxyl radicals (‘OH) and lipid alkoxyl radicals generated from bio-Fenton and Haber–Weiss reactions (Equations 1 and 2), which require hydrogen peroxide (H₂O₂) and transition metals (presumably redox-active iron, Fe²⁺) as primary sources. Iron-dependent enzymatic lipid peroxidation is processed in a controlled manner via the lipoxygenase (LOX) family and cytochrome P450 oxidoreductase (POR) [26, 33].

Fenton (1) and Haber–Weiss (2) Reaction:

\[
\text{Fe}^{II} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{III} + \text{HO}^\cdot + \text{HO}^\cdot \quad (1)
\]

\[
\text{Fe}^{III} + \text{O}_2^\cdot \rightarrow \text{Fe}^{II} + \text{O}_2 \quad (2)
\]

Free Fe²⁺ can be uptaken via the transferrin receptor (TFRC) and stored in ferritin cages, which undergoes ferroptosis-associated lysosomal degradation and releases labile iron to promote ferroptosis (Figure 1). Besides, iron sources are also stored intracellularly as heme and iron-sulfur clusters (ISCs). The cysteine desulfurase nitrogen fixation 1 (NFS1) that participates in ISC synthesis was found to be associated with ferroptosis susceptibility. The activation of NFS1 in cancer cells leads to a reduced level of labile iron, thereby losing the ferroptosis sensitivity [36]. CDGSH iron-sulfur domain-containing protein 1 (CISD-1) and CDGSH iron-sulfur domain-containing protein 2 (CISD-2) regulate the intracellular iron homeostasis; the overexpression of CISD-1 and CISD-2 can result in ferroptosis resistance against class I FINs [37, 38]. Recent studies reported that cancer cells could develop a natural protective mechanism in response to ferroptotic stress, including GPX4 inhibition and cell detachment from the extracellular matrix (ECM) (Figure 2). Upon the RNA-Seq screening of protein expression induced by pro-ferroptotic stimuli, prominin-2, a pentaspanin protein, was identified to be positively correlated with cell resistance to ferroptosis [19]. The key finding is that prominin-2 expression promoted the formation of ferritin-containing multivesicular bodies (MVBs) and exosomes that eliminate iron sources out of cells [19]. It was determined that the blockage of the prominin-2-mediated iron export pathway could restore the ferroptosis sensitivity to GPX4 inhibitors.

**The anti-oxidative pathways regulate ferroptosis susceptibility**

Lipid peroxidation and ferroptosis induced by ROS have been explored to address the chemoresistance of cancer cells [18, 39]. Considering the fact that cancer cells often suffer...
from a variable degree of oxidative stress during the tumor progression and therapeutic stimuli, but maintain at low lipid peroxidation rates, indicating their capacity to develop defensive antioxidant mechanisms [4, 19, 20, 23, 40, 41]. To date, some significant pathways have been identified that participate in maintaining oxidative homeostasis of membrane lipids and that can negatively regulate ferroptosis sensitivity, including: 1) the system \( \text{XC}^{-}/\text{GSH}/\text{GPX4} \) axis; 2) the Mevalonate-ferroptosis suppressor protein 1 (MVA-FSP1) axis; 3) the \( \text{GTP cyclohydrolase 1-Tetrahydrobiopterin} \) axis; 4) the peroxisome-ether-phospholipid axis; 5) acyl-CoA synthetase long-chain family member 3-monounsaturated fatty acids (ACSL3-MUFA) axis; and 6) the Liver kinase B1-AMP-activated protein kinase (LKB1-AMPK) axis. Therefore, current findings should collectively catalyze further studies to support the development of novel therapeutics against ferroptosis resistance.

GPX4 is a crucial glutathione peroxidase found in cellular membranes that repair preoxidized PL-PUFAs (Figure 1). In this regard, intracellular GSH is an antioxidant tripeptide that works as an essential cofactor to reduce lipid hydroperoxides [4]. *De novo* GSH synthesis requires a constant cysteine supply, which can either be produced from the transsulfuration pathway or imported from extracellular cysteine via system \( \text{XC}^{-} \) in exchange for intracellular glutamate [42]. Interference of system \( \text{XC}^{-} \) activity through class I FINs (erastin, erastin2, 5-octyl D-glutamate, sorafenib, and sulfasalazine) blocks cysteine uptake, resulting in insufficient GSH production [5]. Moreover, GSH-depleting agents
(class I FINs) such as acetaminophen, n-acetyl-4-benzoquinone imine (NAPQI), and buthionine sulfoximine (BSO) can also lead to oxidative stress-induced ferroptosis [4, 9]. Alternatively, ferroptosis can be induced by direct inhibition of phospholipid peroxidase activity of GPX4 through covalent binding with the selenocysteine active site; these are known as class II FINs (e.g., altretamine, ML-162, ML-210, RSL3, and withaferin A) [10, 12, 26].

However, some tumor cells develop protective mechanisms enabling evasion from ferroptosis. The nuclear factor erythroid 2-related factor 2 (NRF2) is a key regulator in tumor cells that controls the expression of antioxidant proteins in response to ferroptosis stress [20]. Activation of the NRF2 pathway confers ferroptosis resistance to class I/II FINs. The inhibition/knockdown of NRF2 in hepatocellular carcinoma can increase sensitivity to erastin, sorafenib, and BSO [43]. Similarly, the acquired resistance to artesunate in head and neck cancer (HNC) cells could be reversed by NRF2 inhibition [21]. Moreover, inhibiting the NRF2 pathway in cisplatin-resistant cells (HN3R) and RSL3-resistant (HN3-rslR) cells yields enhanced sensitivity to RSL3 [22]. Thus, NRF2 could be a potent target for sensitizing class I/II FINs-resistant tumor cells.

In addition to the system XC−/GSH/GPX4 axis, the MV A-FSP1 axis is also crucial for GPX4 maturation and biosynthesis of coenzyme Q_{10} (CoQ_{10}). GPX4 is a selenoprotein that requires the incorporation of selenocysteine (Sec) into its active site via the isopentenylation of tRNAsec uses isopentenyl pyrophosphate (IPP), a central intermediate in the MV A pathway (Figure 1) [44]. Besides, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a rate-controlling enzyme of the MV A pathway (Figure 1). Thus, HMG-CoA reductase inhibitors, often known as statins, act by blocking the GPX4 and CoQ_{10} biosynthesis via inhibition of IPP formation [45]. Like the oxime-containing molecule FIN56, several compounds promote the degradation of GPX4 and interfere with the CoQ_{10} biosynthesis [13]. Reduced CoQ_{10} (ubiquinol) is an endogenous antioxidant produced by the MVA-FSP1 pathway [46]. Of note, the antioxidant capacity of ubiquinol is maintained by membrane-tethered ferroptosis-suppressor protein 1 (FSP1), an NADH-dependent CoQ oxidoreductase that reduces CoQ_{10} to ubiquinol. CoQ_{10} synthesis and FSP1 machinery given their central role in the MVA-FSP1 axis, which has been validated as a second antioxidant system that acts fully independent of the X_{c}−/GSH/GPX4 axis [41, 47], suggests that FSP1 is a vital ferroptosis resistance factor that can be targeted to sensitize specific ferroptosis-resistant cancer cells [41]. FSP1 can be activated by a nuclear receptor protein, PPARα, dependent on upstream negative regulatory MDM2/MDMX complex, in a p53-independent manner (Figure 1) [48].

In a related vein, the ubiquinol level can also be reinforced by GTP cyclohydrolase-1 (GCH1) generated tetrahydrobiopterin (BH_{4}) upon ferroptosis-induction (Figure 1) [49, 50]. Moreover, the production of BH_{4} enables lipid remodeling with its direct antioxidant effect to protect specific PUFA-PLs from oxidative degradation [49, 50]. Therefore, the GCH1-BH_{4} axis could be considered a potential target for those cancer cells resistant to GPX4 inhibition.

With respect to the lipid metabolism associated with ferroptosis susceptibility, it was determined that the polyunsaturated ether phospholipids (PUFA-ePLs) act as a key pro-ferroptotic substrate for lipid peroxidation and is closely associated with ferroptosis execution [23]. The biosynthesis of PUFA-ePLs is driven by peroxisome biogenesis [51, 52], which sufficiently controls sensitivity to ferroptosis (Figure 3) [23]. In this sense, the peroxisome-ether-phospholipid axis has been proposed to explain how adaptive evasion of ferroptosis induction can occur in some carcinoma cells. The study has uncovered that the initial ferroptosis-sensitive cells could adapt to survive under oxidative stress through significant downregulation
of PUFA-ePLs [23]. This suggests that the modulation of peroxisome and PUFA-ePLs levels could help promote the ferroptotic process in cancer treatment.

Besides, a recent study has explored a new potent ferroptosis inducer dihomo-γ-linolenic acid (DGLA), an exogenous PUFAs that appears to have a unique pro-ferroptotic effect distinct from other exogenous PUFAs, including arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid, which did not induce Fer-1-sensitive ferroptosis alone [53]. The mechanism of DGLA-induced ferroptosis was proposed through the direct modulation of membrane phospholipid acylation (Figure 3) [53]. Notably, the evidence indicates that endogenous ether lipids (mainly plasmalogens) could act as antioxidant molecules to protect both Caenorhabditis elegans and cancer cells from ferroptosis induced by exogenous DGLA [53, 54]. These findings imply that endogenous biosynthesis of plasmalogens can play distinct roles in response to specific ferroptotic stimuli. Moreover, a ferroptosis-resistant cell state can also be triggered by exogenous and endogenous MUFAs [29, 53, 55]. Acyl-CoA synthetase long-chain family member 3 (ACSL3) is required for MUFAs activation. Once activated, MUFAs can incorporate into the plasma membrane and reduce the ferroptotic sensitivity by altering PUFA composition of the plasma membrane [55].

Interestingly, recent studies found that ATP depletion in cancer cells can activate AMP-activated protein kinase (AMPK), a ubiquitous cellular energy sensor that confers resistance to ferroptosis by negatively regulates acetyl-CoA carboxylase (ACC) and PUFA biosynthesis (Figure 3) [56–58]. It has been confirmed that a key serine-threonine kinase encoded by LKB1 activates the AMPK through direct phosphorylation, which is one of the frequently mutated tumor suppressors in several human cancers [59]. Further investigation indicated that LKB1-AMPK negatively regulates the rate-limiting enzyme ACC1 during the PUFA biosynthesis upon the energy stress [57]. Collectively, the study concluded that LKB1-AMPK-dependent ACC1-mediated fatty acid biogenesis is essential for ferroptosis execution. In line with these findings, gaining a more in-depth understanding of the regulation in lipid metabolic dynamics and membrane lipid composition is essential for developing novel strategies against ferroptosis resistance.

**Perspectives**

The discovery of ferroptosis offered a tremendous therapeutic opportunity in cancer treatment, and its practical effectiveness in clinical settings is steadily progressing. Several Food and Drug Administration-approved drugs, including altretamine, sorafenib, artesunate, and artemisinin, have been recognized with pro-ferroptotic effect. A number of FINs have been developed and verified in preclinical cancer models [60]. However, recent studies have identified new metabolic pathways that confer resistance to cancer cell ferroptosis induction, particularly the role of lipid metabolism in the regulation of ferroptosis sensitivity [19, 23, 53, 57]. A deeper understanding of mechanisms driving ferroptosis-sensitive state remains further investigation. In this regard, targeting three hallmark features (i.e., PUFA-PLs, redox-active iron, and antioxidant system) of ferroptosis provides effective routes to eradicating aggressive malignancies. Aside from ferroptosis mechanistic studies, interdisciplinary approaches with the integration of nanomedicine, immunotherapy, and radiotherapy hold additional opportunities to promote ferroptosis-based therapeutic approaches toward clinical translation.

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