



REVIEW ARTICLE

Ferroptosis as a novel form of regulated cell death: Implications in the pathogenesis, oncometabolism and treatment of human cancer

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KEYWORDS

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Abstract The treatment of cancer mainly involves surgical excision supplemented by radiotherapy and chemotherapy. Chemotherapy drugs act by interfering with tumor growth and inducing the death of cancer cells. Anti-tumor drugs were developed to induce apoptosis, but some patient's show apoptosis escape and chemotherapy resistance. Therefore, other forms of cell death that can overcome the resistance of tumor cells are important in the context of cancer treatment. Ferroptosis is a newly discovered iron-dependent, non-apoptotic type of cell death that is highly negatively correlated with cancer development. Ferroptosis is mainly caused by the abnormal increase in iron-dependent lipid reactive oxygen species and the imbalance of redox homeostasis. This review summarizes the progression and regulatory mechanism of ferroptosis in cancer and discusses its possible clinical applications in cancer diagnosis and treatment.

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Introduction

Cell death is the irreversible termination of cell life processes and plays a significant role in organism survival and development.^{1,2} There are many common forms of cell death, including necrosis and apoptosis.^{3–5} Initially, only apoptosis was thought to be the only programmed form of cell death, but as research progressed, it was found that there are more types, including cysteine protease-1 (Caspase-1)-dependent apoptosis and receptor-interacting protein kinase 1 (RIPK1)-dependent necroptosis.^{6–9}

Ferroptosis is a mode of iron-dependent non-apoptotic cell death, discovered in 2012, that is characterized by the excessive accumulation of lipid peroxides and reactive oxygen species (ROS).¹⁰ Ferroptosis differs from apoptosis, necrosis, and other types of cell death by the morphology, genetics, metabolism, and molecular biology of the dying cell.^{11,12} Studies have found that ferroptosis can inhibit the proliferation of tumor cells in liver, pancreatic, kidney, prostate, and breast cancer.^{13–17} Therefore, inducing ferroptosis is expected to provide a new method for anti-tumor therapy and bring new hope to cancer patients. On the other hand, activation of ferroptosis can accelerate the progression of acute kidney injury and neurodegenerative diseases such as Alzheimer's disease.^{18,19} Other studies have similarly suggested the therapeutic benefits of inhibiting ferroptosis in patients who have an ischemia/reperfusion injury (IRI). Induction of ferroptosis could therefore concerningly lead to further neuronal damage, cardiomyopathy, and renal tubular cell death in brain, heart, and kidney IRI's respectively.²⁰

Cell death escape is one of the most important characteristics of cancer cells, regardless of the cell death type.^{21–23} Therefore, a detailed study on the molecular mechanisms and biochemical characteristics of a cell death pathway can provide a set of possible targets for cancer treatment.²⁴ Ferroptosis, in particular, has attracted a lot of attention in the research community because of its important role in the development, progression, and multidrug resistance of cancers.^{25–27} This study reviews the definition and characteristics of ferroptosis and summarizes its regulation mechanism in cancers as well as its possible clinical application in anti-tumor therapy.

Forms of regulated cell death

There are two distinct cell death types, regulated cell death (RCD) and accidental cell death (ACD).^{28,29} RCD relies on specialized molecular mechanisms and can be modulated (delayed or accelerated). It is also not unique to multicellular life forms.²⁸ RCD occurs during the development or regeneration of single-cell eukaryotes (yeast and *Dictyostelium discoideum*) as well as certain prokaryotes (*Escherichia coli*). In these cases, RCD is also known as programmed cell death (PCD), which refers to completely physiologic forms of RCD.^{12,28} ACD is the instantaneous and disorganized death of cells under physical (high pressure, temperature, or osmotic force), chemical (extreme pH changes), or mechanical (shear force) conditions.²⁹

Historically, cell death has been divided into three different categories according to the observed changes in cell morphology (Table 1). This includes type I cell death (apoptosis) where the morphology of cells is characterized by cytoplasmic contraction, chromatin consolidation, nuclear fragmentation, plasma membrane bubbling, and finally formation of undisrupted regular vesicles (commonly known as apoptotic bodies). Type II cell death (autophagy) involves extensive cytoplasmic vacuolization, which is followed by autophagosome-lysosome fusion and cell content degradation. Finally, type III cell death (necrosis) occurs without the involvement of autophagosomes and lysosomes, and its endpoint instead is phagocytic clearance.^{30,31}

The discovery of ferroptosis

In 2003, Dolma et al investigated the mechanism of action of the anti-tumor drug erastin in RAS-mutated cancer cells and found a distinct pattern of cell death that differs from apoptosis. However, the new mechanism was not yet named at that time.³² In 2008, Yang and Stockwell identified two compounds, Ras-selective lethal 3 (RSL3) and Ras-selective lethal 5 (RSL5), which cause cell death in a manner very similar to that of erastin. The resultant cell death process is inhibited by iron chelators and antioxidants, which suggests that it is influenced by iron and ROS.³³ Moreover, when Dixon et al treated a human fibrosarcoma HT-1080 cell line

Table 1 Characteristics of the classic forms of cell death.

	Apoptosis	Autophagy	Necrosis
Inducing factors	Altered expression of genes under physiological conditions	Nutrient deficiency or hormones	Extreme physicochemical conditions or strong pathological stimuli
Cell morphology at the initial stages of cell death	Cells become smaller in size	Cells produce vacuoles	Cells become swollen and deformed
Cell membrane	Preserves continuity, forms apoptotic bodies	Intact structure	Damaged structure
Organelles	Intact structure	Contained in autophagosomes, digested in lysosomes and vacuoles	Deformed or swollen
Chromatin	Condensed or fragmented	No typical features	Collapsed
DNA	Broken into fragments of varying size	Randomly degraded	Randomly degraded
Specific enzymes	Caspase	Lysosomal enzymes	Lysosomal enzymes
Characteristic proteins	Caspase 3, Bcl-2, Cytochrome c, DAP	PI3K, mTOR, LC3, DAP	DAP
Inflammatory response	Not induced	Can be induced	Can be induced

DNA, deoxyribo nucleic acid; BCL-2, B cell lymphoma-2; DAP, death-associated protein; PI3K, phosphatidyl inositol 3-kinase; mTOR, mammalian target of rapamycin; LC3, light chain 3.

harboring the *N-RAS* mutation with erastin in 2012, analysis by flow cytometry with fluorescent probe labeling showed an increase in the level of cellular lipid ROS 2 h after treatment. After 6 h, the significant increase in the level of ROS was accompanied by an increase in cell death. However, after co-incubation with deferoxamine (DFO), cell death was significantly reduced. Therefore, it can be assumed that the newly discovered cell death mode is directly related to the iron concentration. This was also confirmed by the fact that the addition of ferric ammonium citrate, ferric citrate, ferric chloride, or exogenous iron ions to cell culture as a substitute for other metal ions (Cu^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+}) could increase the level of intracellular ROS as well as the cell death rate.^{10,34} Because Dixon et al found that the increased cell death rate occurred over the period of ROS accumulation and the antioxidants could inhibit this process, they concluded that the examined cell death mode was different from apoptosis, necrosis, and autophagy, and named it ferroptosis.¹⁰

The process and characteristics of ferroptosis

Cystine/glutamate transporter (System x_c^-) is composed of solute carrier family 7 member 11 (SLC7A 11) and solute carrier family 3 member 2 (SLC3A 2) and is an important intracellular antioxidant molecule.^{35,36} System x_c^- is the upstream transporter molecule in the process of ferroptosis, and its main function is to maintain the balance between cystine (Cys–Cys) intake and glutamate (Glu) excretion. The molecule binds Cys–Cys and reduces it to

cysteine (Cys), which is involved in the glutathione (GSH) synthesis pathway.³⁷ Glu reduces ROS and reactive nitrogen species (RNS), which are catalyzed by glutathione peroxidase 4 (GPX 4). Therefore, both Glu depletion and GPX 4 degradation contribute to cellular ferroptosis.¹⁰

Ferroptosis is caused mainly by an abnormal increase in iron-dependent lipid reactive oxygen species and an imbalance of redox homeostasis in cells. Induction of ferroptosis in cancer cells can inhibit its growth, which bring new possibilities for the development of anti-tumor treatments and solves the problem of chemotherapy resistance.^{38–40} There are many differences between ferroptosis and classical cell death modes (apoptosis, autophagy, necrosis, necroptosis, and pyroptosis) in morphology, biochemistry, genetics, and inducers, which are summarized in Table 2.

Metabolic regulatory mechanisms of ferroptosis

Iron, lipids, and amino acids are important regulators of ferroptosis, therefore the metabolism of ferroptosis; therefore, the metabolism of these substances plays a significant role in the process of cell death (Fig. 1).

Iron metabolism

Normal iron metabolism is crucial for the survival of organisms. It is mainly involved in oxygen transport, DNA

Table 2 Differences between ferroptosis and classical forms of cell death.

Forms of cell death	Biochemical characteristics	Characteristics observed under the electron microscope	Regulatory mechanism
Ferroptosis	Participation of iron ions, accumulation of iron-dependent ROS	Increased density of cell outer membrane, decreased volume of mitochondria, absence of mitochondrial cristae, ruptured outer mitochondrial membrane	Iron ion metabolism, iron-dependent ROS metabolic pathways, Ras/Raf/MEK/ERK metabolic pathway
Apoptosis	Activation of the Caspase pathway, cytoplasmic Ca ²⁺ involvement, formation of Bcl-2-bound oligonucleotide nucleosomes and DNA fragmentation, degradation of mitochondrial transmembrane potential protein	Cells shrunken and round, contracted cell membrane with vesicular projections, condensed and fragmented nucleus, chromatin edge collection, degraded DNA, invaginated cell membrane, apoptotic bodies	FasL/FasR, TNF- α /TNFR1, and other exogenous pathways, endogenous pathways such as Bcl-2 and Caspase pathway
Autophagy	Atg, LC3, or p62 as LC3 ligand	Autophagosomes containing cytoplasm and organelles, intact cytoskeleton, degraded organelles such as Golgi apparatus, ribosomes, and endoplasmic reticulum	Atg12-Atg5 and Atg-PE pathways
Necrosis	Consumption of ATP, RIP1, RIP3, and MLKL, release of DAMPs, hyperactivation of PARP1	Swollen cells and organelles, degraded lysosomes, ruptured cell membrane, degraded nuclear chromosome, damaged mitochondria, cell lysis	RIP1 and RIP3, PRR pathways
Necroptosis	Fatal influx of Ca ²⁺ , formation of RIPK1-RIPK3-MLKL complex	Ruptured cell membrane, swollen organelles and dysfunctional mitochondria	TNF-mediated pathway
Pyroptosis	NLRP1b, NLRP3, NLRC4 and AIM2 inflammasome	Cytoplasmic swelling, shrunken cell nucleus, fragmented chromatin, cell membranes with multiple vesicular projections resulting in uneven shear stress and cell membrane rupture	Caspase 1-dependent, accompanied by the release of a large number of proinflammatory factors

ROS, reactive oxygen species; Ras, rat sarcoma virus; Raf, rapidly accelerated fibrosarcoma; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; BCL-2, B cell lymphoma-2; FasL, fas receptors ligands; FasR, fas receptors; TNF- α , tumor necrosis factor- α ; TNFR1, tumor necrosis factor receptor subtype-1; Atg, autophagy-related genes; LC3, light chain 3; ATP, adenosine triphosphate; RIP1, receptor-interacting protein 1; RIP3, receptor-interacting protein 3; MLKL, mixed lineage kinase domain-like; DAMPs, damage associated molecular patterns; PARP1, poly(ADP-ribose) polymerase 1; PRR, pattern recognition receptor; NLRP1b, nucleotide-binding oligomerization domain-like receptor 1b; NLRP3, nucleotide-binding oligomerization domain-like receptor 3; NLRC4, NLR family CARD domain-containing protein 4; AIM2, absent in melanoma 2.

biosynthesis, the tricarboxylic acid (TCA) cycle, and the electron transport chain as a coenzyme. It also affects the synthesis of ATP.⁴¹ However, excessive levels of iron ions in cells can catalyze the formation of ROS that exhibit metabolic toxicity, leading to cell damage or death.⁴² Iron is also one of the elements necessary for lipid peroxide accumulation and ferroptosis. Therefore, both flux and storage of iron can affect the occurrence of ferroptosis.^{43–45} Studies have shown that Fe³⁺ binds to transferrin receptor 1 (TFR1) anchored in the cell membrane and is then transferred to the endosome. In endosomes, Fe³⁺ is reduced to Fe²⁺, and divalent metal transporter 1 (DMT1) releases it to replenish unstable iron pools in the cytoplasm. Excess iron is coupled to the ferritin light chain (FTL) and the ferritin heavy chain 1 (FTH1), where it is stored in the protein-bound form.^{46,47} Dolma et al found increased levels of TFR1 and decreased levels of

ferritin (FTL and FTH1) in RAS-mutated cells compared with non-mutated controls, which correlated with the occurrence of ferroptosis.¹⁰ This outcome suggests that cells with RAS mutations have increased iron intake and reduced iron storage capacity, and the resultant iron overload can induce ferroptosis.^{48,49} Studies have shown that the use of iron chelating agents (deferramine) inhibit erastin-induced ferroptosis, and supplementation with exogenous iron increases erastin-induced ferroptosis.^{50–52} Therefore, regulation of iron ion levels can significantly affect the occurrence of cellular ferroptosis. TFR1 transports iron from the extracellular environment into the cell, which is a necessary step in ferroptosis. This process is regulated by iron-responsive element binding protein 2 (IREB2), as silencing of the *IREB2* gene is known to reduce the occurrence of ferroptosis.¹⁰ Iron ion levels are also regulated by phosphorylase kinase catalytic subunit gamma 2 (PHKG2),

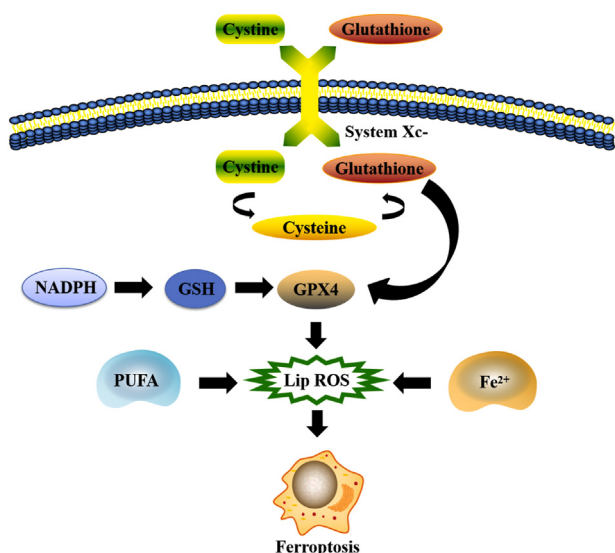


Figure 1 Regulation of iron metabolism, lipid metabolism and amino acid metabolism in ferroptosis.

and silencing of this gene is similarly found to reduce the occurrence of ferroptosis.^{53,54} Autophagy of ferritin also regulates ferroptosis by affecting the level of protein-bound iron in the cell.^{55,56} Several other cellular proteins that affect iron metabolism, such as heat shock protein beta 1 (HSPB1) and CDGSH iron sulfur domain 1 (CISD1), also regulate ferroptosis.^{57,58} HSPB1 can inhibit the circulatory metabolism of transferrin receptor 1 (TFR1), which in turn reduces the content of iron in cells, and therefore reduces the sensitivity to ferroptosis.⁵⁷ On the other hand, CISD1 can increase iron uptake and lipid oxidation in mitochondria, thus enhancing the sensitivity to ferroptosis.⁵⁸ Therefore, the control over iron metabolism and autophagy of ferritin can potentially be used as a way to regulate the occurrence of ferroptosis.

Lipid metabolism

Dyslipidemia is a signal of cell death by ferroptosis, and lipid metabolism is also closely related to cell ferroptosis.⁵⁹ Polyunsaturated fatty acids containing diallyl hydrogen atoms are sensitive to lipid peroxidation, and it is one of the elements necessary for ferroptosis.³³ Membrane phospholipids containing the polyunsaturated fatty acid (PUFA) are enzymatically oxidized to form lipid peroxides which induce cellular ferroptosis.⁶⁰ Moreover, under the further action of excess iron, lipid peroxides continue to generate toxic lipid radicals that act on adjacent PUFA, triggering a new round of lipid peroxidation that further accelerates the process of cellular ferroptosis.⁶¹ The synthesis of PUFA-containing phospholipids requires Acyl-CoA synthetase long-chain family member 4 (ACSL4) and recombinant lysophosphatidylcholine acyltransferase 3 (LPCAT3) as catalysts.⁶² The lack of genes encoding these enzymes prevent PUFA from being inserted into membrane phospholipids, thus preventing the formation of PUFA-containing phospholipids. This inhibits lipid peroxidation and reduces the sensitivity of cells to ferroptosis.⁶³ Lipoxygenase (LOX) is an

active enzyme that catalyzes the formation of lipid peroxides from PUFA-containing membrane phospholipids. Wenzel et al found that phosphatidylethanolamine binding protein 1 (PEBP1) can bind to LOX and change its structure in a way that enhances lipid peroxidation.⁶⁴ On the other hand, it was found that elevated glutathione peroxidase 4 (GPX4) activities in cells could inhibit lipid peroxide formation and avoid cellular ferroptosis. The content and location of polyunsaturated fatty acids determine the degree of lipid peroxidation in cells and thus the degree of ferroptosis.⁶⁵ Lipidomic studies have shown that phosphatidylethanolamine containing arachidonic acid or its derivative, epinephrine, is a key phospholipid that is oxidized and causes ferroptosis.⁶⁶

Amino acid metabolism

Amino acid metabolism is closely related to the regulation of ferroptosis. The key positive regulators in this process are glutamate and glutamine.⁶⁷ Glutamate is exchanged with cystine in a 1:1 ratio by reverse transporter x_c⁻, when concentrations of glutamate are high, this antiporter is inhibited, thereby inducing cellular ferroptosis.¹⁰ Similarly, the degradation product of glutamine can provide substrates for the tricarboxylic acid cycle and lipid biosynthesis. Therefore, the absence of glutamine or the lack of glutamine decomposition inhibits cellular ferroptosis by preventing lipids from being synthesized and thus oxidized.⁵⁵ *In vivo* animal studies have demonstrated that inhibiting glutamine breakdown can reduce ischemia-reperfusion-induced heart and kidney injury as well as cerebral hemorrhage.^{67,68} The first step in the decomposition of glutamine is the conversion of glutamine to glutamate, which is catalyzed by glutaminases GLS1 and GLS2. Although GLS1 and GLS2 are highly similar in structure, only GLS2 has been found to be involved in the regulation of ferroptosis. *GLS2* is a downstream target gene of p53, and upregulation of *GLS2* can promote p53-dependent ferroptosis.⁶⁹ Since ferroptosis is closely related to the mechanism of cell death in damaged tissues, treatment targeting the glutamine degradation pathway may be a promising method for post-injury organ repair.

Inducers and inhibitors of ferroptosis

A variety of substances can induce ferroptosis, including erastin, RSL3 and RSL5, Buthioninesulfoximine (BSO), acetaminophen, lanperisone, salazosulfonypyridine, sorafenib, and artesunate.⁷⁰ There are also substances that are known to inhibit ferroptosis, such as vitamin E, GSH, ferrostatin-1, liproxstatin -1, and iron chelating agents, as shown in Table 3.

Inducers of ferroptosis

The currently known inducers of ferroptosis include molecules regulating the oxidative stress response and iron ion metabolism. erastin, the first discovered ferroptosis inducer, can mobilize voltage-dependent anion channels (VDACs) to trigger mitochondrial dysfunction.⁷¹ Erastin-

Table 3 Inducers and inhibitors of ferroptosis.

Classification	Molecules	Mechanism of action
Inducers	Erastin, sorafenib, p53	Inhibit GPX4 function indirectly by inhibiting the glutathione/cystine reverse transport system and glutathione synthesis
	RSL3, DPI7, DPI10, DPI12, DPI13, DPI17, DPI18, DPI19 FIN56, FINO ₂ , NOX-1, artemisinin	Inhibit GPX4 function directly Promote intracellular ROS accumulation and lead to lipid peroxidation
Inhibitors	PHKG2	Activates ferroptosis by increasing iron availability
	Antioxidants (Vitamin E, Trolox, U0126)	Reduce the intracellular ROS accumulation
	Iron inhibitor and its derivatives, iron chelating agents	Reduce the intracellular content of iron ions
	HSPB1	Reduces the intracellular content of iron ions and lipid ROS
	NRF2, MT1	Block GSH depletion
	Desferrioxamine mesylate	Chelates lysosomal or unstable iron
	Cyclopyridyl ethanolamine	Prevents lipid peroxidation in the cytoplasm

GPX4, glutathione peroxidase 4; RSL3, Ras-selective lethal 3; DPI7, diphenylene iodonium 7; DPI10, diphenylene iodonium 10; DPI12, diphenylene iodonium 12; DPI13, diphenylene iodonium 13; DPI17, diphenylene iodonium 17; DPI18, diphenylene iodonium 18; DPI19, diphenylene iodonium 19; FIN56, ferroptosis-inducing 56; FINO₂, 1,2-dioxolane; NOX-1, NADPH oxidase 1; ROS, reactive oxygen species; PHKG2, phosphorylase kinase G2; HSPB1, heat shock protein family B member 1; NRF2, nuclear factor erythroid 2-related factor 2; MT1, membrane type 1; GSH, glutathione.

induced ferroptosis can happen by the production of ROS that damage mitochondria or by direct inhibition of System x_c^- . This process is additionally enhanced by heme oxygenase-1 (HO-1), which increases the availability of intracellular iron ions and induces ROS production.^{72–75} Piperazine erastin, a derivative of erastin, is more soluble and stable in cells than erastin and inhibits the proliferation of human fibroblast HT-1080 cells by inducing ferroptosis.⁷⁶ Imidazole ketone erastin (IKE), an analog of the original drug known for its greater water solubility and metabolic capacity, also has been shown to more effectively induce ferroptosis.⁷⁷ Similar to erastin, lanperisone also induces the production of ROS, causing ferroptosis.⁷⁸ Another molecule, sorafenib, has been widely used in the treatment of advanced liver cancer. It can enhance the toxicity of System x_c^- to liver cancer cells, inhibit their proliferation, and promote the sustained cancer decline.^{79–81} Studies have found that nuclear factor E2-related factor 2 (NRF2) can regulate the production of GSH and activate the p62-KEAP1-NRF2 pathway to achieve antioxidant activity and prevent cellular ferroptosis.⁸² After NRF2 and its target genes were knocked out, the activity of sorafenib was enhanced, which led to the decreased proliferation of liver cancer cells. After p62 knockout, HO-1, NAD(P)H quinone oxidoreductase 1 (NQO1), and FTH1 significantly enhanced the activity of ferroptosis inducers such as sorafenib, erastin, and sulfasalazine.⁸² Small molecule compounds RSL3, DPI2/7/10, and Fin56 can directly inhibit GPX4, and thus increase the production of lipid reactive oxygen species increases, leading to ferroptosis.^{83–85} Inducer RSL5 directly interacts with VDACC2/3 to produce ROS and lead to ferroptosis. Buthionine sulfoximine (BSO) irreversibly inhibits γ -glutamyl cysteine synthase (γ -GCS) activity, thereby reducing GSH synthesis and inhibiting its activity. Similarly,

an overdose of acetaminophen causes an excessive amount of the reactive metabolite *N*-acetyl-*p*-benzoquinone imine to form, thereby reducing GSH stores and inducing ferroptosis.⁸⁶ As mentioned before, excessive extracellular glutamate also inhibits System x_c^- activity, which results in ferroptosis induction.⁸⁷ During the treatment of breast cancer, siramesine can modify the iron content in cells by regulating the expression of transferrin, which can lead to ferroptosis upon iron accumulation. Siramesine also acts as a ferroptosis inducer in conjunction with lapatinib.⁸⁸

Inhibitors of ferroptosis

Regulation of intracellular iron metabolism, increase in GSH levels, increase in GPX4 activity, and direct inhibition of ROS production can all inhibit cellular ferroptosis. Inhibitors of oxidative stress, such as ferrostatin-1 (first-generation ferroptosis inhibitor) rely on aromatic amines to inhibit the accumulation of specific lipid ROS. They have been proven to inhibit ferroptosis induced by erastin and RSL3.⁸⁹ However, second-generation (SRS11-92) and third-generation ferroptosis inhibitors (SRS16-86) showed more stable metabolism and significantly improved resistance to tissue damage than first-generation ferroptosis inhibitors.⁹⁰ As mentioned earlier, ferroptosis can be inhibited by up-regulating NRF2 expression. It can also be prevented by using zileuton to inhibit 5-LOX activity and reduce ROS production.⁹¹ Recently, it was discovered that ferroptosis suppressor protein 1 (FSP1) also acts as a ferroptosis inhibitor. FSP1 (formerly known as apoptosis-inducing factor mitochondrial 2), is recruited to the plasma membrane through the process of myristoylation, where it then acts as an oxidoreductase that reduces coenzyme Q10 (CoQ). This

process creates a lipophilic radical-trapping antioxidant (RTA) that stops the formation of lipid peroxides, thereby inhibiting ferroptosis.⁹² In addition, trolox, ebselen, tocopherol, vitamin E, losroxin A, pepstatin methyl ester, butyl hydroxytoluene, and ammonium chloride all inhibit ferroptosis by interfering with lipid peroxidation pathway.^{93–95}

Inhibitors regulating iron metabolism effectively inhibit erastin-induced ferroptosis by optimally reducing excessive iron uptake. For example, heat shock protein beta 1 (HSPB1) is involved in the uptake of iron into cells and can affect the expression of its own gene *HSPB1* by inhibiting the expression of heat shock transcription factor-1 (HSF-1), thus inhibiting erastin-induced ferroptosis.⁵⁷ The reduction of *TFR1* expression by shRNA also inhibits ferroptosis induced by erastin. Iron chelators, such as DFO and deferoxamine mesylate, inhibit ferroptosis by reducing the amount of iron available as the electron donor in the process of lipid peroxidation, thus preventing the production of ROS and negatively regulating erastin-induced ferroptosis in human cervical cancer cells, osteosarcoma cells, and prostate cancer cells.^{70,89,96–98}

Ferroptosis plays an important role in inhibiting tumorigenesis, and cancer cells reduce cell death by inhibiting ferroptosis. solute carrier family 7 member 11 (SLC7A11) is highly expressed in human cancers, and its overexpression inhibits the ferroptosis of cancer cells. When SLC7A11 has been inhibited, ferroptosis can be induced.^{99,100} Studies have shown that GPX4 plays an important role in the fight against cellular ferroptosis by reducing phospholipid hydrogen peroxide and inhibiting lipoxygenase-mediated lipid peroxidation.^{101,102} GPX4 uses GSH as a cofactor to catalyze the reduction of lipid peroxides, protect cells and cell membranes from peroxides, and inhibit the occurrence of cellular ferroptosis.¹⁰³ Studies have shown that mitoneer (CISD1) protein, an iron-containing mitochondrial outer membrane protein, is used to regulate mitochondrial iron uptake and respiratory function, and can negatively regulate the ferroptosis of cancer cells.^{58,104}

Clinical implications

Biomarker

ACSL4 is a member of the long-chain acyl coenzyme A (acyl-CoA) synthase (ACSL) family. It catalyzes the synthesis of arachidonic acid CoA and adrenal acid CoA and is involved in the synthesis of phosphatidylethanolamine, phosphatidylinositol, and other negatively charged membrane phospholipids.^{58,62} As an important gene of the lipid metabolism pathway, knockout of the *ACSL4* gene in mouse and human cells can effectively reduce the cell mortality induced by erastin and RSL3.^{105,106} *ACSL4* expression was significantly downregulated of ferroptosis-resistant cells (such as LNCaP and K562) compared with that in ferroptosis-sensitive cells (such as HepG2 and HL60). Using CRISPR/Cas9 to perform genome-wide screening and genomic microarray analysis, *ACSL4* was found to be an executive factor of ferroptosis, making it a promising biomarker of the process.⁶²

Reduced nicotinamide adenine dinucleotide phosphate (NADPH) is a coenzyme of GSH reductase and therefore

plays an important role in maintaining intracellular GSH levels.¹⁰⁷ Classical inducers of ferroptosis (erastin, RSL3, and Fin56) were studied in 12 different cell lines, including fibrosarcoma, osteosarcoma, and striated cancer cells.^{108,109} In said cell lines, intracellular NAD(H) and NADP(H) levels were significantly reduced, and the formation of lipid ROS was identified.¹¹⁰ The consumption of NAD(P) (H) represents the degree of lipid peroxidation, and its level may be a useful biomarker for monitoring the susceptibility of cells to ferroptosis inducers.^{111,112}

The gene encoding cyclooxygenase-2 (COX-2) is a widely-used biomarker of ferroptosis in cancer cells induced by erastin or RSL3.⁶⁶ *In vitro* experiments have shown that ferroptosis can be detected by various methods involving the measurement of cell activity, iron content, and ROS levels. However, it is still difficult to prove the existence of ferroptosis *in vivo*.⁷²

Clinical treatment

Suitable ferroptosis inducers or inhibitors can play a useful therapeutic role in different types of cancers. As a newly discovered type of programmed cell death, ferroptosis kills cancer cells utilizing a unique mechanism and signaling pathway, especially in cancer cells that are not sensitive to death by chemoradiotherapy and apoptosis.¹¹³ For example, pancreatic cancer cells are resistant to chemotherapy drug-induced apoptosis but are sensitive to artemisinin-induced ferroptosis.¹¹⁴ In addition, dihydroartemisinin has been proven to induce ferroptosis in head and neck squamous cell carcinoma.¹¹⁵ Lapatinib can induce ferroptosis in breast cancer cells, and acetaminophen can induce ferroptosis in liver injury and hepatotoxicity.^{88,116,117} Low-level laser irradiation followed by gallic acid treatment was found to treat melanoma cancer cells by inactivating GPX4 activity and thereby inducing ferroptosis.¹¹⁸ The occurrence of ferroptosis in gastric cancer cells was limited by restoring intracellular GSH level, activating GPX4 expression, inhibiting lipid peroxidation, and little ROS accumulation.^{119,120} Moreover, several molecules, such as RSL3, RSL5, artesunate (ART), docosahexaenoic acid (DHA), and a series of small-molecule ferroptosis-inducing agents (FINs), have been shown to promote ferroptosis in cancer cells.^{121–123} Among 60 examined cancer cell lines from eight different sources from the National Cancer Institute (NCI), kidney cancer cells and leukemia cells were more sensitive to the ferroptosis inducer erastin than the cells derived from the other six cancer lines (lung, colon, central nervous system, melanocytes, ovaries, and breast).¹⁰⁹ In addition, erastin has been proven to enhance the therapeutic effects of temozolomide, cisplatin, cytarabine, and doxorubicin in specific cancers.¹²⁴ Traditional Chinese medicine (TCM) has played a significant role in the clinical treatment of cancers, but its mechanism is still unclear.^{125,126} Recently, it has been found that baicalein can be used as a new ferroptosis inhibitor, and its effect is significantly better than that of typical ferroptosis inhibitors (such as Fer-1 and ferriamine mesylate).¹²⁷ This provides a basis for further studies comparing the effects of TCM on ferroptosis with the effects of related drugs. Finally, as mentioned earlier,

ACSL4 is a promising biomarker for ferroptosis induction. Doll et al recently discovered that this enzyme is significantly expressed in a subset of triple-negative breast cancer cell lines. With a new possibility of inducing ferroptosis into breast cancer cells with this biomarker, this finding provides a promising outlook for potential treatments of this deadly disease.⁶²

Conclusion and future directions

Ferroptosis is a newly discovered form of cell death. At present, the research on ferroptosis is still in the primary stage, and there are many problems to be solved. Further research on the mechanism of cellular ferroptosis could be helpful for the clinical treatment of cancers, and therefore has great developmental potential. Research on the molecular mechanism of ferroptosis as well as potential inducers and inhibitors of the process are likely to be the main focus of future studies. In addition, drugs that are currently widely used in the clinic, such as sorafenib, may find new applications in the context of ferroptosis, which will have an important value in basic research and clinical applications.

Conflict of interests

The authors declare that they have no competing interests.

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