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Metabolic networks in ferroptosis (Review)

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Abstract. Ferroptosis is an iron-dependent and peroxidation-driven form of cell death associated with multiple metabolic disorders and disrupted homeostasis. A number of metabolic processes and homeostasis are affected by ferroptosis. The molecules that regulate ferroptosis are involved in metabolic pathways that regulate cysteine exploitation, glutathione state, nicotinamide adenine dinucleotide phosphate function, lipid peroxidation and iron homeostasis. The present review summarizes the metabolic networks involved in ferroptosis based on previous studies, and discusses the function of ferroptosis in pathological processes, including cancer. Finally, the clinical significance of ferroptosis is highlighted, to provide evidence for further studies.

Contents

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Abbreviations: ACSL4, acyl-CoA synthetase long-chain family member 4; CARS, cysteinyl tRNA synthetase; CBS, cystathionine-β-synthase; CGL, cystathionine-γ-lyase; FTH1, ferritin heavy chain 1; GPX, glutathione peroxidase; GPX4, glutathione peroxidase 4; GSH, glutathione; G6PD, glucose-6-phosphate dehydrogenase; IPP, isopentenyl pyrophosphate; L-ROS, lipid-based reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate; NCOA4, nuclear receptor coactivator 4; NOX, NADPH oxidase; NRF2, nuclear factor E2-related factor 2; PGD, 6-phosphogluconate dehydrogenase; PUFAs, polyunsaturated fatty acids; RCD, regulated cell death; Sec, selenocysteine; Sec-tRNA$^{[Ser]Sec}$, selenocysteine transfer RNA; Xc-, glutamate/cysteine antiporter

Key words: ferroptosis, cysteine metabolism, lipid peroxidation, nicotinamide adenine dinucleotide phosphate, iron homeostasis

1. Introduction

Regulated cell death (RCD) is essential for the development of living organisms and the maintenance of homeostasis. As a novel form of RCD, ferroptosis has begun to attract increased attention (1-4). Ferroptosis is an iron-dependent and peroxidation-driven form of cell death that is distinct from apoptosis, necrosis and other types of cell death in terms of morphology, genetics, metabolism and molecular biology (2,5-10).

Apoptosis is characterized by morphological changes that include chromosome shrinkage, chromatin condensation and cytoplasmic fragment formation (2,11). Positive regulators of apoptosis include pro-apoptotic B-cell lymphoma-2 (Bcl-2) family proteins, for example BCL2 associated X, apoptosis regulator and BCL2 antagonist/killer 1. Negative regulators of apoptosis include Bcl-2 and other anti-apoptotic Bcl-2 family proteins. Concerning the process of necrosis, loss of plasma membrane integrity occurs instead of the mitochondrial shrinkage and increased mitochondrial membrane density that occurs during ferroptosis. Regulators involved in necrosis include receptor interacting serine/threonine kinase 1 (RIPK1), receptor interacting serine/threonine kinase 3 and mixed lineage kinase domain like pseudokinase (12,13).

The regulators involved in apoptosis and necrosis are not required for ferroptosis. Ferroptosis is regulated by glutathione peroxidase 4 (GPX4), a lipid repair enzyme (14,15), and is associated with the following group of genes: Iron responsive element binding protein 2, citrate synthase, ribosomal protein L8 and ATP synthase, H+ transporting, mitochondrial Fo complex subunit C3 (subunit 9) (2). Ferroptosis is a type of cellular sabotage that results in cell death, whereas apoptosis, pyroptosis and necroptosis are considered types of programmed cell death (16). Ferroptosis is not blocked by carbobenzoxy-valyl-alanyl-aspartyl-(O-methyl)-fluoromethylketone, an inhibitor of caspase-dependent apoptosis and pyroptosis, or by necrostatin-1, an inhibitor of RIPK1-dependent necroptosis (17,18).

Ferroptosis is driven by inhibition of extracellular cysteine uptake and inactivation of the function of GPX4 (5,15). These processes lead to the depletion of polyunsaturated fatty acids (PUFAs) in lipid bilayers and the accumulation of lipid-based reactive oxygen species (L-ROS) (2,3,7,9,15). Furthermore, iron metabolism and cellular iron abundance simultaneously impact the onset of ferroptosis. The inducers and inhibitors of ferroptosis are categorized and summarized in Table I (2,5,7,10,15,18-24).

Ferroptosis is associated with multiple physiopathological processes and human diseases, particularly the occurrence and progression of multiple types of cancer. Previous studies have revealed that hepatocellular carcinoma (HCC), renal cell carcinoma, diffuse large B-cell lymphoma, pancreatic carcinoma and ovarian cancer cells are susceptible to ferroptosis (15,25,26). The metabolic peculiarities of ferroptosis vary among different types of cancer cell.

The present review provides a comprehensive overview of studies concerning the metabolic networks involved in ferroptosis in cancer cells.

2. Metabolism and ferroptosis

Cysteine is critical for ferroptosis. Cellular cysteine is primarily obtained by extracellular cysteine uptake through the glutamate/cysteine antiporter (Xc^-) (6,27,28). In addition to extracellular uptake, certain mammalian cells are able to use methionine as a sulfur donor to synthesize *de novo* cysteine through the trans-sulfuration pathway (6,28-30). However, mammalian cells normally depend on only one of these patterns as the major source of cysteine. The trans-sulfuration pathway provides a compensatory source of cysteine when the uptake pattern is inhibited.

The Xc^- system consists of a 12-pass transmembrane protein transporter solute carrier family 7 member 11 (SLC7A11) and a single-pass transmembrane regulatory protein solute carrier family 3 member 2. Acting as a glutamate-cystine antiporter, inhibition of the Xc^- system may lead to depletion of the intracellular cysteine pool, one of the molecular events that induces ferroptosis (2,7,21,28). As a classic inducer of ferroptosis, erastin suppresses the glutamate-cystine antiporter (21). Upregulation of SLC7A11 prevents cells from erastin-induced ferroptosis, while downregulation of SLC7A11 inhibits the growth of cancer cells during erastin treatment (2,21).

As another source of cysteine, the trans-sulfuration pathway is catalyzed and regulated by cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CGL) (31) (Fig. 1). Genome-wide siRNA screening has revealed that silencing of cysteinyl tRNA synthetase (CARS) suppresses erastin-induced ferroptosis (10,28). CBS and CGL are upregulated in CARS-deprieved cells, and metabolites accumulate in the trans-sulfuration pathway following erastin treatment (6,10,32). These results support the hypothesis that the transsulfuration pathway is a regulator of ferroptosis resistance, compensating for cysteine depletion caused by the inhibition of cysteine uptake.

GSH biosynthesis is connected to cysteine and GPX4. In the 1970s, deprivation of Cys2 was revealed to lead to marked depletion of GSH and the promotion of cell death (3,33), suggesting that cysteine uptake may be the limiting factor for GSH biosynthesis. Several subsequent pharmacological studies of glutamate- or erastin-induced ferroptosis further demonstrated that decreased GSH levels triggered by cysteine deprivation may induce the initiation of oxidative stress and ferroptotic cell death (2,3,7,15,20,28). GSH biosynthesis is critical for protecting cells from oxidative damage, and the cysteine-GSH pathway is one of the most pivotal upstream mechanisms for the execution of ferroptosis.

GSH biosynthesis is critical for the functional activity of GSH-dependent enzymes, including selenium glutathione peroxidase (GPX). GPX uses the thiol group in GSH as an electron donor and affects the cellular antioxidant reaction (15,28). Inactivation of GPX4 induced by GSH depletion increases intracellular lipid peroxides, resulting in ferroptosis (10,15).

The mevalonate pathway is crucial for GPX4 maturation in ferroptosis. In addition to its dependence on GSH, GPX also relies on cysteine metabolism for maturation. GPX, a typical selenoprotein, uses its catalytic center selenocysteine (Sec) during defense against antioxidants. During the process of GPX maturation, Sec transfer RNA (Sec-$tRNA^{[Ser]Sec}$) is one of the key regulatory elements modulated by isopentenyl pyrophosphate (IPP), a product of the mevalonate pathway (Fig. 1) (34-36). Serving as a primary source of IPP, the mevalonate pathway is a crucial signaling network for GPX4 maturation and ferroptosis induction. FIN56 is a novel inducer of ferroptosis discovered during the study of nonapoptotic cell death (37). Unlike erastin, FIN56 treatment does not result in GSH depletion, but causes GPX4 loss at the post-translational level and the decrease of mevalonate-derived lipophilic antioxidants, indicating that FIN56-induced ferroptosis is modulated through the mevalonate pathway (37). Thus, GPX4 maturation may link the mevalonate pathway and ferroptosis.

Previous studies investigating the functions of statins in the prevention of obesity-associated cardiovascular diseases have demonstrated that 3-hydroxy-3-methylglutaryl-coenzyme A reductase serves as a target of statins in the mevalonate pathway (38). Preclinical studies have demonstrated the pro-apoptotic effects of statins (39,40). Furthermore, human prostate cancer PC3 cells treated with atorvastatin undergo autophagy, whereas simvastatin leads to the induction of apoptosis in HCT116 colorectal cancer cells and renal cell carcinoma cells (41). Statins also downregulate the mevalonate pathway and block the biosynthesis of cellular isoprenoids, including IPP, which are responsible for the post-translational modification of Sec-$tRNA^{[Ser]Sec}$ and the synthesis of GPX4 (6,35,42). Although there is no experimental evidence demonstrating the link between statins and ferroptosis, statins downregulate the mevalonate pathway, which is a crucial signaling event for GPX4 maturation. Thus, ferroptosis may be a form of statin-induced cell death. Further research is required to investigate the association between statins and ferroptosis.

Dual effects of nicotinamide adenine dinucleotide phosphate (NADPH) on sensitivity to ferroptosis. NADPH, the predominant reducing agent in organisms, participates in a number of metabolic reactions. GSH is dehydrogenated to form glutathione disulfide, which is in turn reduced to GSH by glutathione reductase in the presence of NADPH (43). Given the functions of GSH, the synthesis of NADPH is important in resistance to peroxidation-induced damage during ferroptosis.

NADPH is produced by the pentose phosphate pathway, which is catalyzed by glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (PGD; Fig. 1). Several studies have demonstrated that nuclear factor E2-related factor 2 (NRF2) targets the genes encoding G6PD and PGD (44-46). Silencing of NRF2 and these

Table I. Inducers and inhibitors of ferroptosis.

A, Inducers

Author, year	Type	Molecules and drugs	Mechanism	(Refs.)
Hayano *et al*, 2016; Yang and Stockwell, 2008	I	Erastin Glutamate Buthioninesulfoximine Acetaminophen Sorafenib Sulfasalazine	Inhibit the glutamate/cysteine antiporter system and block glutathione synthesis, indirectly inhibiting GPX4	(10) (18)
Hayano *et al*, 2016; Yang *et al*, 2014	II	(1S,3R)-RSL3, DPI19, DPI18, DPI17, DPI13, DPI12, DPI10, DPI7 Altretamine	Directly inactivate GPX4 without glutathione decrease	(10) (15)

B, Inhibitors

Author, year	Type	Molecules and drugs	Mechanism	(Refs.)
Dixon *et al*, 2012	I	Cycloheximide	Suppress protein synthesis and lipid peroxidation	(2)
Magtanong *et al*, 2016		β-mercaptoethanol		(5)
Skouta *et al*, 2014		Trolox		(7)
Hayano *et al*, 2016		Baicalein		(10)
Yang *et al*, 2014		Zileuton Liproxstatin-1 Ferrostatin-1		(15)
Dixon *et al*, 2012	II	Deferoxamine	Chelate lysosomal iron or the labile iron pool in the cytoplasm to protect against lipid peroxidation	(2)
Hayano *et al*, 2016		Ciclopirox olamine		(10)
Yang *et al*, 2008		2,2-BP		(18)
Louandre *et al*, 2013				(19)
Dixon *et al*, 2014				(20)
Xie *et al*, 2016				(21)
Kurz *et al*, 2006				(22)
Barradas *et al*, 1989				(23)
Soupene and Kuypers, 2008				(24)

GPX4, glutathione peroxidase 4.

enzyme-associated genes causes HCC cells to be sensitized to the ferroptosis inducers erastin and sorafenib (47). Consequently, NRF2 functions as a negative regulator of ferroptosis in liver cancer cells, participates in NADPH production and subsequently affects GSH function, which is essential for the initiation of ferroptosis.

Notably, NADPH depletion sensitizes fibrosarcoma HT-1080 cells to ferroptosis inducers, indicating that NADPH is negatively associated with ferroptosis sensitivity (48). However, well-established studies concerning the NADPH oxidase (NOX) protein family have demonstrated that NADPH provides electrons for NOX to generate superoxide from oxygen (49), which may promote the ferroptosis process. Furthermore, inhibition of the pentose phosphate pathway partially rescues Calu-1 cells from ferroptosis (2). The results of these studies support the contradictory function of NADPH in ferroptosis. Further investigations are required to determine the function of NADPH, as an inducer or an inhibitor of ferroptosis.

Ferroptosis is induced by lipid peroxidation. Cell lines selected for Xc^- system inhibition resistance have been demonstrated to overexpress aldo-keto reductase family members, which detoxify oxidative lipid fragments (6,20). Thus, the lipid fragments may be downstream products generated from cysteine depletion. This discovery provides insights into the potential associations between cysteine and lipid metabolism and the mechanisms of lipid peroxidation in ferroptosis (Fig. 1).

Two lipid metabolism-associated genes, acyl-CoA synthetase long-chain family member 4 (*ACSL4*) and lysophosphatidylcholine acyltransferase 3 (*LPCAT3*), encode enzymes required for the acylation and insertion of PUFAs

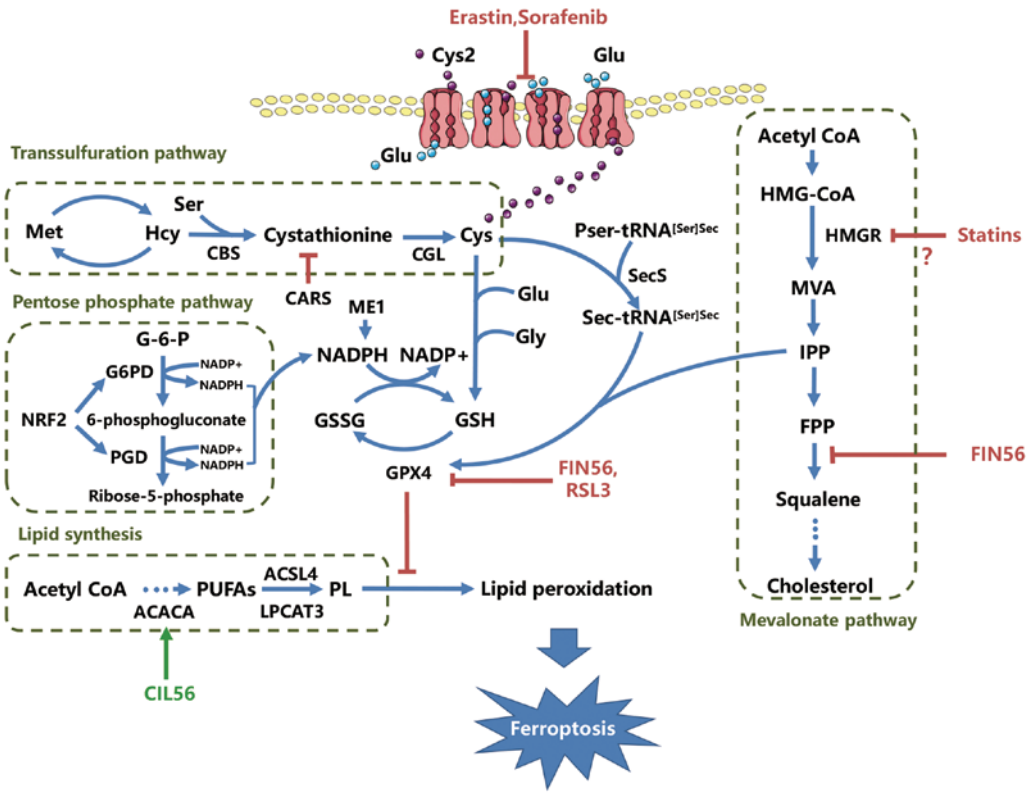

Figure 1. Ferroptosis-associated metabolic networks. Several metabolic pathways are involved in ferroptosis in cells. The trans-sulfuration and pentose phosphate pathways are crucial for Cys and GSH maintenance, which promote ferroptosis. GSH synthesis involves selenoproteins, including glutathione peroxidase 4. The mevalonate pathway, which sustains isopentenyl pyrophosphate/farnesyl pyrophosphate for selenoproteins, may also be a major cellular redox regulator. Furthermore, lipid peroxidation is regulated by metabolic enzymes in lipid synthesis. Inducers of ferroptosis are indicated in red, and inhibitors of ferroptosis are indicated in green. The question marks indicate the stochastic relationships between molecules. GSH, glutathione.

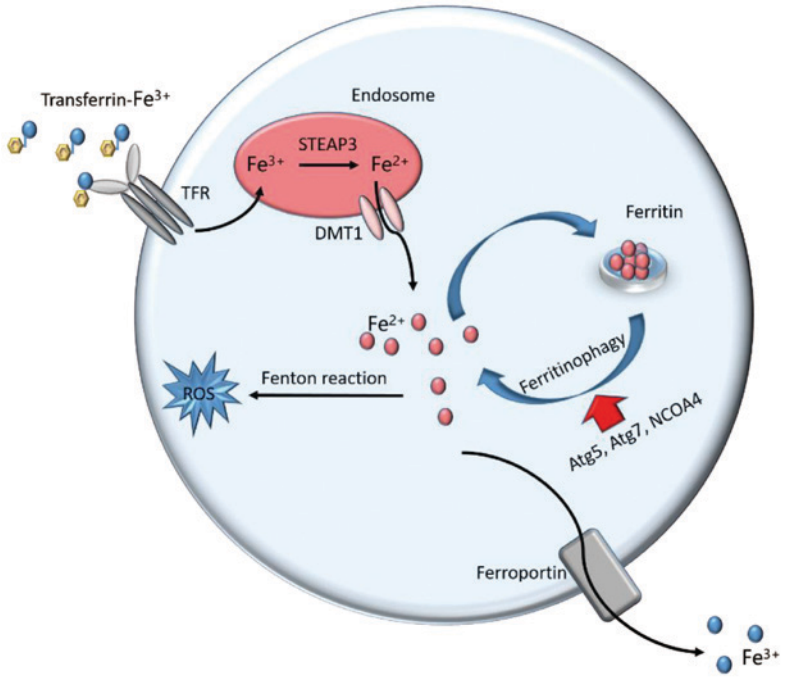

Figure 2. Iron metabolism in ferroptosis. Cellular iron homeostasis is dependent on the coordination of iron uptake, export, utilization, and storage. Extracellular Fe^{3+} binds to transferrin and is taken up into cells through TFR1. The freed Fe^{3+} is reduced to Fe^{2+} by STEAP3 metalloreductases in the endosome. Divalent metal transporter 1 mediates the transport of Fe^{2+} from the endosome into a labile iron pool in the cytoplasm. The labile iron is exported via the membrane protein ferroportin to maintain plasma iron levels. Alternatively, or in parallel, excess iron from the labile iron pool is stored in ferritin heteropolymers (ferritin heavy chain 1 or ferritin light chain), a redox-inactive form of iron, to protect cells and tissues from iron-mediated damage. Notably, the autophagic degradation of ferritin, a process known as ferritinophagy, increases labile iron levels and contributes to ferroptosis. Fe^{3+}, ferric iron; Fe^{2+}, ferrous iron; TFR1, transferrin receptor 1; STEAP3, six-transmembrane epithelial antigen of prostate 3; DMT1, doublesex and mab-3 related transcription factor 1; ROS, reactive oxygen species; Atg5, autophagy related 5; Atg7, autophagy related 7; NCOA4, nuclear receptor coactivator 4.

into membrane phospholipids, respectively (24,50). A previous study demonstrated that deletion of *ACSL4* and *LPCAT3* prevents RSL3 and ML162-induced ferroptosis in KBM7 cells (51). Thus, inhibition of phospholipid synthesis may suppress ferroptotic cell death. Furthermore, initiation of ferroptosis results in the depletion of PUFAs in lipid bilayers and the accumulation of L-ROS and lysophospholipids (7,9,15). Lysophospholipids and oxidized PUFAs are products of glycerophospholipids, and are formed via a degradation reaction catalyzed by phospholipase A2 (9). These results suggest that the PUFAs provided by glycerophospholipids are required as substrates for lipid peroxidation during ferroptosis.

PUFAs oxidized and cleaved from glycerophospholipid backbones are subsequently degraded, producing a series of toxic metabolites in ferroptosis. The peroxidation of PUFAs in membranes generates toxic lipid hydroperoxides, resulting in the formation of lethal lipid radicals in the presence of ferrous iron, while inhibiting GPX4 (7,15,52). Lipid radicals react with adjacent PUFAs in lipid membranes and induce lipid peroxidation in ferroptosis. However, the precise pathways through which lipid peroxidation directly or indirectly leads to ferroptosis remain unclear.

Ferroptosis is associated with 'ferritinophagy'. Iron serves a pivotal function in various fundamental metabolic processes due to its role as an auxiliary factor of proteins (53). As ferroptosis is inhibited by an iron chelator, desferrioxamine B-methane sulfonate (DFO), the association between intracellular iron and ferroptosis has become a topic of interest.

Although the mechanisms through which cellular iron facilitates ferroptosis remain unclear, cellular iron homeostasis is recognized as a key factor in ferroptotic cell death (Fig. 2). An excess of iron is stored in ferritin heavy chain 1 (FTH1) and ferritin light chain, and genetic inhibition of FTH1 promotes erastin and sorafenib-induced ferroptosis in HCC cells (47). Furthermore, increased transferrin receptor 1 and decreased ferritin expression occur in ferroptosis-sensitive cells with Ras mutations (18). These results suggest that the abundance of free iron contributes to the induction of ferroptosis via increased iron intake and decreased iron storage.

The introduction of iron chelators further supports the involvement of iron in the process of ferroptosis. Iron chelators are divided into lipophilic iron chelators (including 311, ciclopirox olamine, and 2,2-BP) and membrane impermeable iron chelators (including DFO), which inhibit ferroptosis via diverse mechanisms (2,15,18). DFO chelates lysosomal iron, which should be present at a different location in the cell, promoting L-ROS production. In addition, lipophilic iron chelators are able to cross membranes and chelate the labile iron pool, which is critical for the fragmentation and peroxidation of PUFAs (22,23,52). Excess active iron donates electrons to generate ROS based on the Fenton reaction, promoting lipid peroxidation and the initiation of ferroptosis (54).

Ferritin, a form of stored labile iron, is an antioxidant that inhibits iron-mediated lipid peroxidation (55). Autophagic degradation of ferritin (a process known as ferritinophagy) contributes to ferroptosis via increased labile iron levels in fibroblasts and cancer cells, supporting the association between autophagy and ferroptosis (56). At the genetic level, multiple autophagy-related genes have been identified as positive regulators of ferroptosis. Genetic inhibition of autophagy-related 5 and autophagy-related 7 abrogates the accumulation of labile iron and prevents erastin-induced ferroptosis (56,57). In addition, knockdown of nuclear receptor coactivator 4 (NCOA4), a ferritinophagy cargo receptor, also inhibits ferritinophagy and ferroptotic cell death. In contrast, overexpression of NCOA4 increases ferritinophagy and promotes ferroptosis (56,57). These results suggest that autophagy activation leads to ferritinophagy and promotes ferroptosis by regulation of intracellular iron homeostasis.

3. Conclusion

Ferroptosis is an aberrant metabolic process involving amino acids, lipids, NADPH and microelements. Metabolism of these substances serves a crucial function in cell proliferation and differentiation. However, cysteine depletion, GPX4 inactivation, and iron overload cause cells to experience metabolic stress or ferroptotic cell death. Ferroptosis is characterized by a metabolic imbalance and the perturbation of redox homeostasis. The abundance of the amino acid cysteine and the existence of NADPH, which is primarily generated by the pentose phosphate pathway, are essential for the antioxidant function of GPX4. Furthermore, the inactivation of GPX4 contributes to lipid peroxidation and results in the induction of ferroptosis. The metabolic processes in ferroptosis are not independent, but are instead a part of an intricate metabolic network.

Multiple physiopathological processes and human diseases are involved in ferroptosis. Several types of cancer cell are susceptible to ferroptosis; thus, ferroptosis may represent a novel anticancer therapy. In acute kidney failure, hemorrhagic stroke and nephrotoxic folic acid induce acute kidney injury, and inhibitors of ferroptosis (for example, ferrostatin-1) reduce the damage caused by cell sabotage. Ferrostatin-1 preserves renal function and decreases injury, oxidative stress and tubular cell death in mice with nephrotoxic folic acid-induced acute kidney injuries (17,58,59). Therefore, ferrostatin-1 may have a prophylactic effect in these non-neoplastic diseases.

Ferritinophagy has also been demonstrated to serve as a bridge between ferroptosis and autophagy. Autophagy exerts positive effects in the regulation of ferroptosis. The mechanisms through which autophagy is connected to ferroptosis and through which this relationship is regulated are important. Thus, further studies are needed to determine whether there are any other metabolic processes involved in the association between ferroptosis and autophagy, and whether there is a link between other forms of RCD and ferroptosis.

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