Mechanisms of modulation of ferroptosis and its role in central nervous system diseases

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Abstract

Ferroptosis is a new form of programmed cell death characterized by intracellular iron-dependent accumulation of lipid peroxide and primarily associated with iron metabolism, glutathione-dependent pathway, and Coenzyme Q10-dependent pathway. Recent studies demonstrate that ferroptosis is associated with central nervous system (CNS) diseases, such as stroke, Parkinson's disease, Alzheimer's disease, and Huntington's disease. This review summarizes the key regulatory mechanisms of modulation of ferroptosis and its role in CNS diseases. These updates may provide novel prospective on therapeutical agent developments against CNS diseases.

Mechanisms of modulation of ferroptosis and its role in central nervous system diseases

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ABSTRACT

Ferroptosis is a new form of programmed cell death characterized by intracellular iron-dependent accumulation of lipid peroxide and primarily associated with iron metabolism, glutathione-dependent pathway, and Coenzyme Q_{10}-dependent pathway. Recent studies demonstrate that ferroptosis is associated with central nervous system (CNS) diseases, such as stroke, Parkinson's disease, Alzheimer's disease, and Huntington's disease. This review summarizes the key regulatory mechanisms of modulation of ferroptosis and its role in CNS diseases. These updates may provide novel perspective for the development of therapeutical agents against CNS diseases.

KEYWORDS

Ferroptosis, Lipid metabolism, Iron metabolism, Glutathione peroxidase 4, Coenzyme Q_{10}, Central nervous system diseases

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ABBREVIATIONS

ROS, reactive oxygen species;

DFO, deferoxamine;

PUFA, polyunsaturated fatty acid;

PL, phospholipid;

RPL8 , ribosomal protein L8;

IREB2 , iron response element binding protein 2;

ATP5G3 , ATP synthase F0 complex subunit C3;

CS , citrate synthase;

TTC35 , tetratricopeptide repeat domain 35;

ACSF2 , acyl-CoA synthetase family member 2;

HSPB1, heat shock protein β-1;

PTGS2 , prostaglandin-endoperoxide synthase 2;

Fer-1, ferrostatin-1;

CQ, chloroquine;

CNS, central nervous system;

LOOH, lipid hydroperoxide;

CoQ_{10}, coenzyme Q_{10};

4-HNE, 4-hydroxy-2-nonenal;

MDA, malondialdehyde;

SFA, saturated fatty acid;

MUFA, monounsaturated fatty acid;

AA, arachidonic acid;

AdA, adrenic acid;

PE, phosphatidylethanolamine;

ACSL4, acyl-CoA synthetase long-chain family member 4;

LPCAT3, lysophosphatidylcholine acyltransferase 3;

TZD, thiazolidinedione;

TRO, troglitazone;

PIO, pioglitazone;

ROSI, rosiglitazone;

LOX, lipoxygenase;

L*, pentadienyl radical;

*OH, hydroxyl radicals;

LO*, alkoxy groups;

HO_2*, hydroperoxyl radicals;

LOO*, peroxy radical;

RTA, radical trapping antioxidant;

Lip-1, liproxstatin-1;

Tf, transferrin;

TfR, transferrin receptor;

DMT1, divalent metal transporter 1;

LIP, labile iron pool;

FT, ferritin;

FTH1, ferritin heavy chain 1;

FTL, ferritin light chain;

NCOA4, nuclear receptor coactivator 4;

FPN, ferroportin;

GPX4, glutathione peroxidase 4;

Se, selenium;

GSH, glutathione;

L-OH, lipid alcohol;

RSL3, Ras-selective lethal 3;

Sec, selenocysteine;

Glu, glutamate;

Cys, cysteine;

Gly, glycine;

BSO, buthionine sulfoximine;

GCL, glutamate-cysteine ligase;

IPP, isopentenyl pyrophosphate;

SQS, squalene synthase;

HMG-CoA, 3-hydroxy-3-methyl glutaryl-coenzyme A;

HMGCR, HMG-CoA reductase;

FSP1, ferroptosis suppressor protein 1;

NRF2, nuclear factor erythroid 2-related factor 2;

TXNRD1, thioredoxin reductase 1;

NADK, NAD^+ kinase;

NOX, NADPH oxidase;

DPI, diphenylene iodonium;

TCA cycle, ricarboxylic acid cycle;

Keap1, Kelch-like ECH-associated protein;

NQO1 , quinone oxidoreductase-1;

HO1 , heme oxygenase-1;

ARF, auxin response factor;

FOCAD, Focadhesin;

FAK, focal adhesion kinase;

OHSC, organotypic hippocampal slice culture;

PD, Parkinson's disease;

AD, Alzheimer's disease;

HD, Huntington's disease;

ICH, intracerebral hemorrhage;

MCAO, middle cerebral artery occlusion;

Hb, hemoglobin;

SNpc, substantia nigra pars compacta;

FAC, ferric ammonium citrate;

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine;

Aβ, amyloid-β·

NFT, neurofibrillary tangle;

HP, hippocampi;

FC, frontal cortices;

CDDO, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid;

ARE, antioxidant response element.

1. INTRODUCTION

From organisms to cells, death is the common destiny of life. Conventional cell death removes damaged or harmful cells from organisms. Therefore, cell death is essential for the homeostasis of life. When cell death is over-activated, the body can suffer from many pathological conditions, such as nervous system diseases. Thus, understanding the process of cell death helps to intervene in cell death or survival and develop therapeutical solutions to treat associated diseases.

The major forms of cell deaths are divided into apoptosis, autophagy, and necrosis. Recently, morphological and biochemical criteria have been generated to articulate cell death and mechanisms. New cell death forms are discovered from time to time, such as pyroptosis (Fink et al., 2005) and ferroptosis (Dixon et al., 2012).

In 2012, it was (Dixon et al., 2012) reported that erastin, a small-molecule inducer, induced *RAS* -mutated tumor cell death by overwhelming lipid peroxidation that produced lipid reactive oxygen species (ROS). This cell death form depends on iron rather than other metals, and can be suppressed by iron chelator deferoxamine (DFO). Therefore, such cell death was termed as "ferroptosis" by Dixon and co-workers. Since

then, ferroptosis has drawn a great attention. It was found vital to many pathophysiological conditions, such as nervous system diseases (Derry et al., 2020), ischemia/reperfusion injury (Guan et al., 2019), tumor (Shin et al., 2020) and acute kidney injury (Ma et al., 2020).

Based on the morphological, biochemical, and genetic characteristics, ferroptosis is distinct from other forms of cell death. Ferroptotic cells have smaller mitochondria with reduced crest, condensed membrane density and ruptured outer membranes. The cells are usually rounded up but lack of rupture and blebbing on the plasma membranes, which are the features in apoptotic cells (Xie et al., 2016). The main biochemical characteristic of ferroptosis is iron-dependent over-oxidation of polyunsaturated fatty acids (PUFAs)-containing phospholipids (PLs) on cell membranes. Apoptosis has long benefited from the detection of the cleaved caspase-3. However, it is unclear what are the biomarkers (either transcriptional up-regulation or, post-translational modification of specific cell death effectors or, pore-forming proteins) required for the final execution of ferroptosis. Initially, Dixon and colleagues (Dixon et al., 2012) found that the expression of many genes changed in erastin-induced ferroptosis, including ribosomal protein L8 (*RPL8*), iron response element binding protein 2 (*IREB2*), ATP synthase F0 complex subunit C3 (*ATP5G3*), citrate synthase (*CS*), tetratricopeptide repeat domain 35 (*TTC35*) and acyl-CoA synthetase family member 2 (*ACSF2*). Many genes involved in the regulation of apoptosis and other non-apoptotic cell death are not altered when ferroptosis happened. Subsequently, more genes are being found to be associated with ferroptosis, such as heat shock protein β-1 (*HSPB1*) (Sun et al., 2015), prostaglandin-endoperoxide synthase 2 (*PTGS2*) (Yang et al., 2014) and *p53* (Jiang et al., 2015). Importantly, the expression of *PTGS2* was found to be significantly upregulated in ferroptosis without changing ferroptosis process (Yang et al., 2014). Thus, this gene expression is regarded as a biomarker of ferroptosis.

It is confirmed that ferroptosis depends on the level of lipid ROS. Ferroptosis inhibitors, such as ferrostatin-1(Fer-1) and DFO, prevent cell death by decreasing the lipid ROS. On the other hand, apoptosis inhibitors (e.g., Z-VAD-FMK), necrosis inhibitors (e.g., necrostain-1) and autophagy inhibitors (e.g., chloroquine) cannot suppress ferroptosis.

In this review, we summarize the mechanisms of modulation of ferroptosis and its role in central nervous system (CNS) diseases, and propose the possible strategies for finding new ferroptosis regulators.

2. THE KEY REGULATORY MECHANISMS OF FERROPTOSIS

Ferroptosis is intracellular excessive lipid peroxidation and the metabolic disorders of its product lipid hydroperoxides (LOOHs)*per se* (Figure 1A). Iron catalyzes LOOHs over-production that destroys the intracellular redox balance and triggers cell death. Therefore, LOOHs are key to ferroptosis. Suppressing LOOH level can stop ferroptosis. Lipid peroxidation and metabolic disorders of intracellular LOOHs are mainly related to iron metabolism (Figure 1B), glutathione (GSH)-dependent pathway (Figure 1C) and Coenzyme $Q_{10}(CoQ_{10})$-dependent pathway (Figure 1D). The detailed ferroptosis mechanisms are still to be articulated.

There are several hypotheses on the mechanisms: (1) Lipid peroxidation and ROS over-production destroy cell membrane integrity through damaging and perforating the cell membranes (Agmon et al., 2018); (2) LOOHs are decomposed into toxic aldehydes, such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA), which crosslink and dysfunction the proteins required for cell viability, result in cell death (Angeli et al., 2017; Zhong et al., 2015).

2.1 Lipid metabolism

Compared with saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), PUFAs are easier to be oxidized. This is because the double bond near the *bis* -allyl methylene group in PUFAs can weaken the hydrogen bonding energy of the methylene group, resulting in its sensitivity to dehydrogenation and subsequent oxygenation (Else, 2017). A recent study demonstrated that, when containing the two types of PUFAs arachidonic acid (AA) and adrenic acid (AdA), the PLs especially phosphatidylethanolamines (PEs)

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on the cell membranes, are more susceptible to be oxidized. This can lead ferroptosis eventually (Kagan et al., 2017).

Lipid peroxidation process consists of several steps, (1) in intracellular environment, acyl-CoA synthetase long-chain family member 4 (ACSL4) converts free AA and AdA to AA-CoA and AdA-CoA, (2), AA-CoA and AdA-CoA are then inserted to PLs by esterification reaction under the catalysis of lysophosphatidylcholine acyltransferase 3 (LPCAT3) (Magtanong et al., 2018). Consequently, easily oxidized membrane PLs are synthesized, which are required for the lethal lipid peroxidation and ferroptosis.

Therefore, at least ACSL4 and LPCAT3 are essential for modulating ferroptosis. The ACSL4 specific inhibitors thiazolidinediones (TZDs) include troglitazone (TRO), pioglitazone (PIO) and rosiglitazone (ROSI) (Figure 2A). They were reported to suppress ferroptosis in mouse embryonic fibroblasts (Doll et al., 2017). Knockdown of Lpcat3 could also make mouse lung epithelial cells and embryonic cells more resistant to ferroptosis (Kagan et al., 2017). When ACSL4 or LPCAT3 are inhibited, the available substrates of lipid peroxidation are reduced and the lipid peroxidation is suppressed. ACSL4 and LPCAT3 are promising targets against ferroptosis or other peroxidation related diseases.

Lipoxygenases (LOXs) are iron-containing enzymes for cell membrane PLs oxidation, which are non-heme dioxygenases that catalyze the double oxygenation reaction of PUFAs. Different subtypes of LOXs catalyze the dioxygenation of PUFAs at different positions. Baicalein, a naturel bioactive compound, was reported to inhibit ferroptosis by suppressing 12/15-LOX (Li et al., 2019).

The first step of LOXs catalysis is to abstract an unstable hydrogen from the *bis* -allyl position at a PUFA to form a pentadienyl radical (L*) (Kuhn et al., 2015). This step can also be accomplished by an auto-oxidation reaction independent of LOXs. Highly reactive substances such as hydroxyl radicals (*OH), alkoxy groups (LO*) and hydroperoxyl radicals (HO$_2$*) can take a hydrogen atom from the *bis* -allyl position of a PUFA (Angeli et al., 2017). Subsequently, molecular oxygen is added to the carbon-centered radicals to yield a peroxy radical (LOO*). The LOO* can abstract a hydrogen atom from an adjacent PL to generate a LOOH and a new L*. As a result, this free radical chain reaction continues to propagate and generates more LOOHs (Yin et al., 2011). Additionally, LOOHs can be oxidized by Fe^{2+}: the O-O bonds are broken and yield LO*. The LO* participates in the free radical chain reaction, destroys the adjacent PUFAs directly, and causes cell membrane damage and ferroptosis (Gaschler et al., 2017).

With Fe^{2+}, Fenton reaction converts H$_2$O$_2$ to *OH radicals, which propagate free radical chain reactions (Ayala et al., 2014). Radical trapping antioxidants (RTAs) provide electrons to neutralize free radicals (Hassannia et al., 2019). This suppresses the propagation of lipid peroxidation and act as ferroptosis inhibitors. Such ferroptosis inhibitors include Fer-1 (Dixon et al., 2012), liproxstatin-1 (Lip-1) (Angeli et al., 2014) and α-tocopherol (vitamin E) (Kajarabille et al., 2019) (Figure 2B).

2.2 Iron metabolism

The demand for iron is a defining characteristic of ferroptosis. Since Fe^{2+} catalyzes Fenton reaction, and also is an essential component of ROS-producing enzymes such as LOXs and NADPH oxidase, iron affects lipid peroxidation and cellular sensitivity to ferroptosis. Increasing the content of free Fe^{2+} in cells advances their sensitivity to ferroptosis. Conversely, iron chelators (Figure 2C) and other substances that can reduce the concentration of intracellular iron are able to inhibit ferroptosis. Under physiological conditions, cellular iron homeostasis is regulated through iron uptake, storage and export.

Transferrin (Tf)-mediated iron transport is the most important way of cellular iron uptake. It can transport Fe^{3+} from the place where Fe^{3+} is absorbed and stored to the iron-requiring site of the body. The Tf carrying Fe^{3+}is recognized by the transferrin receptor (TfR) on the cell membranes and endocytosed into the cells. Fe^{3+} is released from the Tf in the acidic environment of endosomes and reduced to Fe^{2+} by ferrous reductase. Afterwards, Fe^{2+} is transported to the cytoplasm through divalent metal transporter 1 (DMT1) on the endosomal membranes (Ji et al., 2015). The free Fe^{2+} forms a labile iron pool (LIP) and plays its physiological or pathological roles. Inhibiting the iron uptake could reduce the level of LIP and suppress

ferroptosis. For example, either immuno-depletion of Tf in serum or RNAi of *TfR* could significantly inhibit ferroptosis in mouse embryonic fibroblasts (Gao et al., 2015).

The excess Fe^{2+} in the cells would be stored in ferritin (FT) to maintain the content of iron under normal physiological conditions. FT is a hollow globular protein shell composed of two types of subunits: ferritin heavy chain 1 (FTH1) and ferritin light chain (FTL) (Harrison et al., 1996). Each FT can store about 4500 Fe^{3+} in the form of $Fe_2O_3 \cdot nH_2O$ (Islam et al., 1989). Oncogene-*RAS*-harboring cancer cells are more sensitive to ferroptosis, partly because *RAS* can down-regulate the expression of FTH1 and FTL, increasing intracellular LIP (Yang et al., 2008). Recent studies indicated that nuclear receptor coactivator 4 (NCOA4)-mediated ferritinophagy played a crucial role in the regulation of iron levels. When available iron in cells is scarce, NCOA4 would recognize and bind to FTH1, and then recruit FT to autophagosomes. With the formation of autolysosomes, FT complexes enter lysosomes and are degraded, subsequently Fe^{3+} stored in these complexes would be released and supply the LIP. This process is also necessary for the execution of ferroptosis. Silencing the expression of NCOA4 by RNAi knockdown significantly inhibited ferritinophagy, thereby suppressing ferroptosis in mouse embryonic fibroblasts (Gao et al., 2016).

In addition, excess cellular Fe^{2+} can also be exported through ferroportin (FPN) on the cell membranes, which is the only known vertebrate iron efflux pump (Bogdan et al., 2016). It was reported that knockdown of *Fpn* in neuroblastoma cells could increase the accumulation of iron-dependent lipid ROS, and thereby accelerate erastin-induced ferroptosis (Geng et al., 2018).

2.3 GSH-dependent pathway

Glutathione peroxidase 4 (GPX4) is a selenium (Se)-containing enzyme, which plays a central role in the reduction of lipid ROS production. With consumption of two GSH molecules, GPX4 could reduce toxic LOOHs to non-toxic lipid alcohols (L-OHs). However, when GPX4 is deficient or inactive, LOOHs will accumulate to a high level, leading to catastrophic membrane damage. It is currently believed that inhibiting GPX4 by direct or indirect ways is the key to induce ferroptosis.

The ways to inhibit GPX4 directly mainly include covalently binding GPX4 and suppressing its expression. The compound Ras-selective lethal 3 (RSL3, Figure 2D), which can covalently bind to the selenocysteine (Sec) at the active site of GPX4 and inhibit its activity, is a highly effective ferroptosis inducer (Dixon et al., 2012). Knockout of *Gpx4* can promote ferroptosis in mouse embryonic fibroblasts, while overexpression of *Gpx4* made cells more resistant to RSL3-induced ferroptosis (Yang et al., 2014).

Inhibiting GPX4 indirectly mainly involves inhibition of its cofactor GSH production. GSH is synthesized from three amino acids: glutamate (Glu), cysteine (Cys) and glycine (Gly). Among them, the amount of Cys is usually the least in cells, so it is considered to be the key factor limiting the *de novo* synthesis of GSH. Cys exists in its oxidized form cystine outside the cells. Through cystine/Glu antiporter (system X_c^-) on the cell membranes, an extracellular cystine is transported into cells and meanwhile an intracellular Glu is exported. The system X_c^- is a disulfide-link heterodimer consisting of SLC7A11 (xCT) and regulatory subunit SLC3A2 (4F2hc and CD98hc) (Sato et al., 1999). This transport process does not depend on ATP but is driven by the difference in the concentrations of Glu or cystine on both sides of the membranes. Although Cys can be generated via the transsulfuration pathway in some cell types, in many other cell types, at least *in vitro*, the import of cystine via system X_c^- is significant for maintaining the levels of Cys and GSH, and preventing ferroptosis (Magtanong et al., 2018). When this transport is impaired, GSH will be depleted, making GPX4 unable to reduce LOOHs. For example, erastin (Figure 2D), a potent inducer of ferroptosis, is a specific inhibitor of the system X_c^- (Dixon et al., 2012); the deletion of a system X_c^- subunit *Slc7a11* in mice induces ferroptosis and inhibits the growth of pancreatic ductal adenocarcinoma (Badgley et al., 2020); high concentration of extracellular Glu inhibits the import of cystine and promotes ferroptosis, which is termed "oxidative glutamate toxicity" in neurons or neuronal-like cells (Magtanong et al., 2018); cystine deprivation suppresses the growth of head and neck cancer by promoting ferroptosis (Shin et al., 2020). Additionally, inhibiting the synthesis of GSH can also promote ferroptosis. The compound

buthionine sulfoximine (BSO, Figure 3) can induce ferroptosis in retinal pigment epithelium by inhibiting glutamate-cysteine ligase (GCL), a rate-limiting enzyme in *de novo* GSH synthesis (Sun et al., 2018).

2.4 CoQ$_{10}$-dependent pathway

However, inhibiting GPX4 did not activate ferroptosis in some cells (Zou et al., 2019), indicating that there may be pathways independent of GPX4 to regulate ferroptosis.

Researchers have found that the mevalonate pathway could also affect ferroptosis. Isopentenyl pyrophosphate (IPP) is a direct metabolite of mevalonate, which can be used for Sec-tRNA prenylation, CoQ$_{10}$ synthesis and cholesterol biosynthesis (Moosmann et al., 2004). On the one hand, only the prenylated Sec-tRNA can carry Sec to GPX4, complete the synthesis of GPX4 (Warner et al., 2000), and then inhibit ferroptosis (Yang et al., 2016); on the other hand, the reduced form of CoQ$_{10}$(CoQ$_{10}$-H$_2$) is a potent lipophilic antioxidant, which can capture LOO* to prevent the spread of free radical chain reaction and inhibit the production of LOOHs, and meanwhile the CoQ$_{10}$-H$_2$ is oxidized (Bentinger et al., 2007). Therefore, inhibiting Sec-tRNA prenylation and CoQ$_{10}$ synthesis will disrupt GPX4 synthesis and CoQ$_{10}$-H$_2$antioxidant activity respectively, and eventually induce ferroptosis. For instance, FIN56 (Figure 2D) can activate squalene synthase (SQS), a key enzyme in cholesterol biosynthesis,[39] and then suppress Sec-tRNA prenylation and CoQ$_{10}$ synthesis, finally leading to ferroptosis in human fibrosarcoma HT1080 cells (Hassannia et al., 2019; Shimada et al., 2016b). 3-Hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) reductase (HMGCR) is an important enzyme in the mevalonate pathway. Statins, as a type of inhibitors of HMGCR, can promote the lethality of FIN56 (Shimada et al., 2016b).

Unless maintaining in the reduced state, the oxidized CoQ$_{10}$ is unable to inhibit the spread of LOOHs. In 2019, Doll et al. (Doll et al., 2019) and Bersuker et al. (Bersuker et al., 2019) conducted an overexpression screen and a synthetic lethal CRISPR-Cas9 knockout screen, respectively. Both groups revealed that ferroptosis suppressor protein 1 (FSP1) could suppress ferroptosis when knockout or inhibit GPX4. FSP1 is essentially a CoQ$_{10}$oxidoreductase, which utilizes NAD(P)H to catalyze the reduction of CoQ$_{10}$, maintaining the availability of CoQ$_{10}$-H$_2$. Bersuker et al. (Bersuker et al., 2019) found that the expression level of FSP1 was positively correlated with ferroptosis resistance in hundreds of cancer cell lines. Besides, in tumor xenograft mice model, the growth of *GPX4* ^{KO}*FSP1* ^{KO} tumors was suppressed, while *GPX4* ^{KO} tumors grew normally. Through screening nearly 10,000 drug-like compounds, Doll et al. (Doll et al., 2019) identified the first effective FSP1 inhibitor iFSP1 (Figure 2D). HT1080 and mouse Pfa1 treated with iFSP1 were much more sensitive to ferroptosis. In conclusion, by regulating the redox of CoQ$_{10}$, FSP1 acts as an essential component of the non-mitochondrial CoQ$_{10}$ antioxidant system. FSP1-CoQ$_{10}$ is also the first found pathway that is able to compensate for the loss of GPX4 in cells.

2.5 Other factors that regulate ferroptosis

Besides the above pathways, there are many other factors that are involved in the regulation of ferroptosis, including Se, NADPH, thioredoxin, transsulfuration pathway, glutaminolysis and nuclear factor erythroid 2-related factor 2 (NRF2) (Figure 3).

Se is currently recognized as an essential micronutrient beneficial to health. Its beneficial effects are mainly due to its incorporation into selenoprotein in the form of Sec (Friedmann Angeli et al., 2018). Sec is similar to cysteine, in which sulfur is replaced by Se. As mentioned previously, Sec is an important component of GPX4. Therefore, Se influences cellular sensitivity to ferroptosis to some extent. It was reported that Se deprivation significantly increases oxidative stress in cells and their susceptibility to ferroptosis (Cardoso et al., 2017). Moreover, Ingold et al. (Ingold et al., 2018) generated mice with targeted mutation of the active site Sec to Cys of GPX4. They found the *Gpx4*$^{cys/cys}$ mouse embryonic fibroblasts were extremely sensitive to peroxide-induced ferroptosis. In addition to directly participating in the synthesis of selenoproteins, Se may increase the resistance of cells to ferroptosis in indirect ways. A study showed that Se supplement could stimulate transcriptional adaptive program of cells to synthesize more antioxidant selenoproteins, including GPX4 and thioredoxin reductase 1 (TXNRD1), to block ferroptosis (Alim et al., 2019).

NADPH can also modulate ferroptosis by indirectly affecting the activity of GPX4, due to that it is a vital reductant in the process of GSH production. The abundance of basal NADP(H) in cells is positively related to the resistance to ferroptosis. It was reported that knockdown of NAD^+ kinase (NADK), an enzyme that uses NAD^+ to synthesize NADP(H), was able to decrease NADP(H) levels in HT1080 cells and make them more susceptible to ferroptosis inducers (Shimada et al., 2016a). In addition, NADPH oxidase (NOX) family, which is able to decrease the available level of intracellular NADPH, was found to be upregulated in several RAS mutant tumors (Kamata, 2009). The classical NOX inhibitor diphenylene iodonium (DPI) was found to prevent erastin-induced ferroptosis in $KRAS$ mutant Calu-1 non-small cell lung cancer cells (Dixon et al., 2012).

Thioredoxin, a member of cellular antioxidant family (Nordberg et al., 2001), plays an important role in suppressing ferroptosis by maintaining and regulating the redox homeostasis. In a recent study, Llabani et al. (Llabani et al., 2019) performed structural modification of the natural product pleuromutilin and synthesized a series of diverse compounds. Through phenotypic screen and biological evaluation, they discovered that the small molecule ferroptocide could induce lipid peroxidation and ferroptosis in some tumor cell lines. Subsequent studies identified ferroptocide is a covalent inhibitor of thioredoxin. This group also demonstrated that knockdown of thioredoxin led to massive generation of general and lipid ROS in HCT 116 colon cancer cells.

As mentioned above, in some cell types, cysteine can be generated through the transsulfuration pathway rather than system X_c^-. When the intracellular cysteine is insufficient, methionine will act as a sulfur donor and undergo a series of reactions to produce cysteine, which can be used for the synthesis of GSH. Therefore, these cells are not sensitive to ferroptosis induced by inhibitors of system X_c^-. For example, Hayano et al. (Hayano et al., 2016) found that activation of transsulfuration pathway in HT1080 cells could increase their resistance to erastin-induced ferroptosis. Inversely, Wang et al. (Wang et al., 2018) designed and synthesized a compound named CH004 as an inhibitor of cystathionine β-synthase, which catalyzes the first enzymatic reaction in the transsulfuration pathway. They found that compound CH004 triggered ferroptosis in hepatocellular carcinoma HepG2 cells and significantly suppressed tumor growth in a xenograft mice model bearing H22 mouse liver tumor cells.

Glutaminolysis is the metabolism of intracellular glutamine, through which cells use glutamine as a carbon source for the mitochondrial tricarboxylic acid (TCA) cycle as well as a nitrogen source for the synthesis of certain necessary substances. Gao et al. (Gao et al., 2015) found that glutaminolysis was necessary for ferroptosis induced by cystine deprivation: either RNAi knockdown of glutamine influx receptor SLC1A5 or glutaminolysis inhibitor Compound 968 could inhibit cystine deprivation-induced ferroptosis. Mechanistically, the TCA cycle and electron transport chain in mitochondria drive this type of ferroptosis. Inhibition of glutaminolysis could suppress the TCA cycle, the hyperpolarization of mitochondrial membrane potential and the accumulation of lipid ROS, eventually inhibit ferroptosis (Gao et al., 2019). This finding also confirmed the vital role of mitochondria in ferroptosis, which had been long-term controversial.

NRF2, a member of basic leucine zipper transcription factors, is a key regulator of cellular antioxidant response, because its target genes include some antioxidant proteins/enzymes genes. Sun et al. (Sun et al., 2016) revealed the p62-Kelch-like ECH-associated protein (Keap1)-NRF2 antioxidative signaling pathway involved in the ferroptosis resistance in hepatocellular carcinoma cells. They found that $p62$-mediated degradation of Keap1 could promote NRF2 activation. Thus, the genes quinone oxidoreductase-1 ($NQO1$), heme oxygenase-1 ($HO1$) and $FTH1$ regulated by NRF2 protected the cells from ferroptosis by modifying lipid peroxidation and iron metabolism. In addition, it was reported that the cells with higher expression levels of auxin response factor (ARF) were more susceptive to ferroptosis, as ARF could inhibit the ability of NRF2 to activate its target genes, including SLC7A11 (Chen et al., 2017). Recently, a NRF2-Focadhesin (FOCAD)-focal adhesion kinase (FAK) signaling pathway was proposed. FOCAD-FAK signaling was able to make non-small-cell lung carcinoma cells more sensitive to cysteine deprivation-induced ferroptosis, while NRF2 could negatively regulate the pathway (Liu et al., 2020). These findings underlined the role of NRF2 in ferroptosis.

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3. FERROPTOSIS AND CNS DISEASES

With the in-depth study of ferroptosis, its therapeutic potentials have also received widespread attention. It has been widely reported that ferroptosis inducers can potently kill tumor cells and inhibit tumor growth in mouse xenograft tumor models, which indicates that ferroptosis inducers are enormous potential in human cancer treatments (Hassannia et al., 2019; Mou et al., 2019; Shi et al., 2019). For instance, Hassannia et al. (Hassannia et al., 2018) identified withaferin A as a natural ferroptosis inducer in neuroblastoma, which could inhibit the *in vivo* growth and recurrence rate of neuroblastoma xenografts. However, ferroptosis was also found to cause neuronal death in rat organotypic hippocampal slice culture (OHSC) models, showing the harmful pathological effect of ferroptosis (Dixon et al., 2012). Moreover, neuronal cells are more susceptible to ROS toxicity owing to their inherent more membranous fatty acids and less antioxidant enzymes, as well as higher oxidative metabolism (Olmez et al., 2012). Increasing evidence indicates that ferroptosis is a driver in some CNS diseases caused by the dysfunction and death of cerebral neurons, such as stroke, Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD) (Figure 4, Table 1). And ferroptosis inhibitors have exhibited great therapeutic potential for these CNS diseases and more efforts have been made to elucidate the role of ferroptosis in the pathogenesis of these diseases.

3.1 Stroke

In the United States, about 795,000 people experience a new or recurrent stroke each year. Of all strokes, 87% are ischemic stroke and 10% are intracerebral hemorrhage (ICH) stroke (Benjamin et al., 2019). Stroke usually leads to irreparable brain damage and the patients have to suffer from severe sequelae, such as hemiplegia, language impairment and cognitive impairment.

Ischemic stroke is caused by occlusion or contraction of blood vessels that restricts blood supply to certain parts of the brain. Insufficient blood in the brain fails to provide enough oxygen and nutrients to neurons, leading to their activation of the ischemic cascade, which is followed by excitotoxicity, oxidative stress, blood–brain barrier dysfunction, microvascular injury, hemostatic activation, post-ischemic inflammation and eventual cell death. Before ferroptosis was identified, clinical studies had found that iron and oxidative stress could promote brain damage caused by ischemic stroke (Carbonell et al., 2007). Nowadays, more and more evidences prove the relationship between ischemic stroke and ferroptosis. A recent study demonstrated that in acute ischemic stroke model of middle cerebral artery occlusion (MCAO) rats, neuronal ferroptosis was induced by the imbalance of iron metabolism and redox disorder (Lan et al., 2020). While the extract of Naotaifang, a compound Chinese herbal medicine, could suppress ferroptosis through TFR1/DMT1 and SCL7A11/GPX4 pathways, and then played a neuroprotective role on MCAO rats (Lan et al., 2020). After cerebral ischemia, reperfusion is the most effective treatment. However, reperfusion will promote the production of ROS, increasing the damage and worsening the patients' prognosis (Olmez et al., 2012). Therefore, reducing brain ischemia/reperfusion injury is crucial in treating cerebral ischemia. Guan et al. (Guan et al., 2019) found that the natural product carvacrol could inhibit ferroptosis by increasing the expression of GPX4, thereby exerting its protective effects on cognitive dysfunction in gerbils exposed to ischemia/reperfusion. Additionally, Alim et al. (Alim et al., 2019) created a Tat-linked SelP Peptide, which could greatly reduce the cerebral infarct volume caused by ischemia/reperfusion in mice. Mechanistically, this is because the Tat-linked SelP Peptide could block ferroptosis by driving transcriptional response to upregulate GPX4 in neurons.

The incidence of ICH is lower than that of ischemic stroke, but its mortality rate is higher and therapies are fewer. ICH refers to bleeding into the brain due to rupture or leakage of blood vessels, leading to compression of brain tissue and neuronal damage. During this process, hemoglobin (Hb) and heme are released from the lysed erythrocytes. They are considered as neurotoxins because they can release iron and cause neuronal damage and death by enhancing the formation of ROS. The iron in dead cells can also be absorbed by surrounding cells, causing even more catastrophic consequences (Xiong et al., 2014). The iron chelating agents DFO can effectively reduce ICH-induced neuronal damage in rats (Okauchi et al., 2010), and the cell death caused by ICH has the characteristics of ferroptosis *in vivo* and *in vitro* (Zille et al., 2017), all verifying

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that ferroptosis is closely related to ICH brain damage. Li et al. (Li et al., 2017) found that ferroptosis did occur in a mouse model of ICH and contributed to neuronal death. In addition, ferroptosis inhibitor Fer-1 can inhibit Hb-induced neuronal death in OHSCs.

In general, inhibiting ferroptosis can be a promising strategy for the prevention or treatments of stroke. However, no clinical trials that use ferroptosis inhibitors have been reported to treat stroke to date.

3.2 PD

PD is the second most universal age-related neurodegenerative disease. The clinical manifestations include resting tremor, muscle rigidity, gait and posture disorders, which cause great pain and inconvenience to the patients and their families. PD is characterized by the death of dopaminergic neurons, especially those in substantia nigra pars compacta (SNpc) and striatum. The loss of dopaminergic neurons leads to insufficient secretion of dopamine, a pivotal neurotransmitter in the brain. Thus, the nerve conduction is blocked, leading to the symptoms of dyskinesia. Currently, dopamine-based therapies such as levodopa are used in clinic to relieve the motor symptoms in early PD. However, these treatments have no effect on the disease progression. Therefore, it is urgent to develop drugs that can slow or prevent the death of dopaminergic neurons in the brain.

The iron accumulation found on SNpc is one of the characteristics of PD patients, suggesting the link between iron and PD (Moreau et al., 2018). As a strong reducing agent, iron can not only cause ROS production in neurons, but also oxidize dopamine (Guiney et al., 2017). Increasing data have proved that ferroptosis is an important pathway for the cell death of dopaminergic neurons and the occurrence of PD. Zhang et al. (Zhang et al., 2020) treated dopaminergic neurons MES23.5 cells with ferric ammonium citrate (FAC) to simulate the iron overload of PD, as FAC can upgrade ferritin levels in cells. They found ferroptosis occurred in the early stage of cell death, which was also proved in the PD mice. Furthermore, ferroptosis inhibitors also have a significant therapeutic effect on the PD mouse model. In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice, a well-established animal model of PD, Van et al. (Do Van et al., 2016) confirmed that the ferroptosis inhibitor Fer-1 could inhibit the death of dopaminergic neurons. Inspiringly, the results of a phase II clinical trial for PD patients (clinical trial NCT01539837) showed that treatment with iron chelator deferiprone (30 mg/kg) exhibited an improvement in motor symptoms and patients' quality of life (Martin-Bastida et al., 2017).

3.3 AD

AD is the most common type of irreversible dementia and a neurodegenerative disease that often occurs in the elderly. Its histological features are the accumulation of senile plaques composed of amyloid-β (Aβ) and neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau protein in the memory and cognition area of the brain (Citron, 2010). AD is caused by the degradation of memory and cognition neurons, which may be the result of the interaction of genes and environment. The manifestations include behavioral changes, progressive memory loss, delusions, hallucinations and degradation in fine motor skills. Therefore, the patients are unable to live independently, bringing a heavy burden to the patients' families and the society.

Before the definition of ferroptosis, abnormal iron metabolism and lipid peroxidation had been found to participate in the pathogenesis of AD (Obulesu et al., 2011). Evidence indicated that AD patients showed an excessive iron accumulation, which is more than 2 times the iron level observed in normal brains (Lovell et al., 1998). Accumulation of iron can not only prompt the accumulation and/or aggregation of the Aβ and tau protein, but also induces the ROS production in the brain of AD (Yamamoto et al., 2002). Oxidative stress is also reported to be an important pathological phenomenon that begins to appear early in the course of AD (Saito et al., 2019). When redox balance in the brain is impaired, oxidative stress can cause serious damage leading to AD. Moreover, oxidative stress has been reported to exacerbate AD pathology and cognitive dysfunction (Butterfield, 1997). Besides, it was indicated that 12/15-LOX was upregulated in the brain of AD patients, which may be related to the oxidative imbalance of AD (Pratico et al., 2004). Now

increasing evidence implicates that ferroptosis may be involved in neuronal degeneration in AD. According to Morris water maze task, *Gpx4* [KO] mice showed obvious defects in spatial learning and memory function, while ferroptosis inhibitor Lip-1 could ameliorate the neurodegeneration in these mice (Hambright et al., 2017). Besides, a clinical measure on AD patients revealed that the level of GSH was reduced especially in the hippocampi (HP) and frontal cortices (FC), two vital brain regions related to the memory and cognition functions (Mandal et al., 2015).

Therapeutically, iron chelator desferrioxamine has already been conducted a clinical trial in AD in 1991 (Crapper McLachlan et al., 1991). A randomized, multi-center, double-blind Phase II trial using deferiprone for AD patients (clinical trial NCT03234686) is currently ongoing in Australia (Rao et al., 2020). Moreover, as mentioned above, Se can increase the resistance of cells to ferroptosis. It was reported that Se deficiency in the human body was associated with an increased risk of AD (Cardoso et al., 2014). However, in a phaseIIclinical trial, though Se could be delivered into the CNS effectively by selenate, there were no significant effects on cognitive performance outcomes in AD patients. Therefore, the process of ferroptosis participating in AD needs further study, as AD may be the combination of many factors (Cardoso et al., 2019).

3.4 HD

HD is an autosomal dominant neurodegenerative disease caused by the CAG repeat length mutation in the *huntington* gene (Ross et al., 2011). It is characterized by highly selective and severe damage to the corpus striatum, resulting in dance-like movements, dystonia and progressive dementia. The mutant *huntington* may cause oxidative stress and neurotoxicity to the neurons in corpus striatum (Paul et al., 2014), which ultimately results in neuronal dysfunction and neuronal cell death, leading to patients with motor and cognitive impairments. However, the pathological mechanism of HD is complicated and has not been fully elucidated yet.

Some characteristics of ferroptosis have been observed in HD patients and experimental animal models, such as iron accumulation (Dominguez et al., 2016), lipid oxidation (Brocardo et al., 2016), oxidative stress (Pinho et al., 2020) and GSH redox cycle dysregulation (Ribeiro et al., 2012). For example, in R6/2 HD mouse brain, discrete puncta formed by iron accumulation was detected in the periplasmic cytoplasm of striated neurons by synchrotron X-ray fluorescence analysis (Chen et al., 2013). HD patients showed higher plasma lipid peroxidation level and lower GSH level (Klepac et al., 2007). Consistently, Kumar et al. (Kumar et al., 2010) found decreased GSH and GSH-S-transferase in the striatum, cortex and hippocampus in 3-nitropropionic acid-induced HD mouse. These phenomena imply that ferroptosis may play an important role in the pathogenesis of HD.

Stack et al. (Stack et al., 2010) synthesized two triterpenoids derived from 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO). They could reduce oxidative stress in the N171-82Q transgenic mouse model of HD, and improved their rotorod performance and survival. Mechanically, the two triterpenoids activated the NRF2/antioxidant response element (ARE) pathway and upregulated NRF2/ARE induced genes in the brain and peripheral tissues. Therefore, compounds targeting the NRF2/ARE pathway show great promise for the treatment of HD. Some ferroptosis regulators have also been found to work in HD models. For instance, intraventricular delivery of the iron chelator DFO led to an improvement in the motor phenotype of R6/2 HD mice (Chen et al., 2013). Skouta et al. (Skouta et al., 2014) found that the ferroptosis inhibitor Fer-1 and its analogues could prevent cell death in the brain slice model of HD.

4. CONCLUSION AND EXPECTATION

After ferroptosis was defined in 2012, the research on its mechanisms and clinical applications has been a hotspot. The currently known major regulatory mechanisms of ferroptosis involve intracellular lipid metabolism, iron metabolism, GSH-dependent pathway and CoQ_{10}-dependent pathway as stated above. It is possible that certain novel regulators existing in ferroptosis have not been uncovered. And there are still many questions need to be solved regarding the mechanisms of ferroptosis and its relationship with diseases. For example, is there a final biomarker that executes the ferroptotic cell death and what is it? What other

roles does mitochondrion play in ferroptosis except that TCA cycle promotes cystine deprivation-induced ferroptosis? Moreover, since the research on ferroptosis mostly focuses on tumor cells, neuronal cells and mouse embryonic fibroblasts, does ferroptosis occur in other cells?

In addition to cancers, ferroptosis has also been reported to be associated with a variety of CNS diseases, and some ferroptosis inhibitors have achieved inspiring results in related animal models. Nonetheless, the role of ferroptosis in CNS diseases needs further elucidation, and currently there is no definitive evidence linking CNS diseases with ferroptosis in long-term animal model studies. There are not enough evidences that ferroptosis is a drug-like target for CNS diseases. Additionally, the existing small molecular regulators of ferroptosis are still limited, and have some disadvantages such as low stability and poor biocompatibility. Therefore, it is urgent to obtain potent ferroptosis inhibitors with good biocompatibility, strong stability and high safety by target-based or cell-based high-throughput screening, structural modification and other methods. The development of such compounds will be an important direction for the prevention and treatments of some human diseases such as CNS diseases.

In conclusion, the research on ferroptosis and its relationship with CNS diseases would be certainly potential for further understanding of the pathogenesis of these diseases and discovery of more effective therapeutic targets, although there are still many unsolved issues in the field. Since no effective therapeutic strategies toward CNS diseases, blockade of ferroptosis may be of value in the treatment of CNS diseases. Further insights into research related to ferroptosis are now likely to emerge rapidly.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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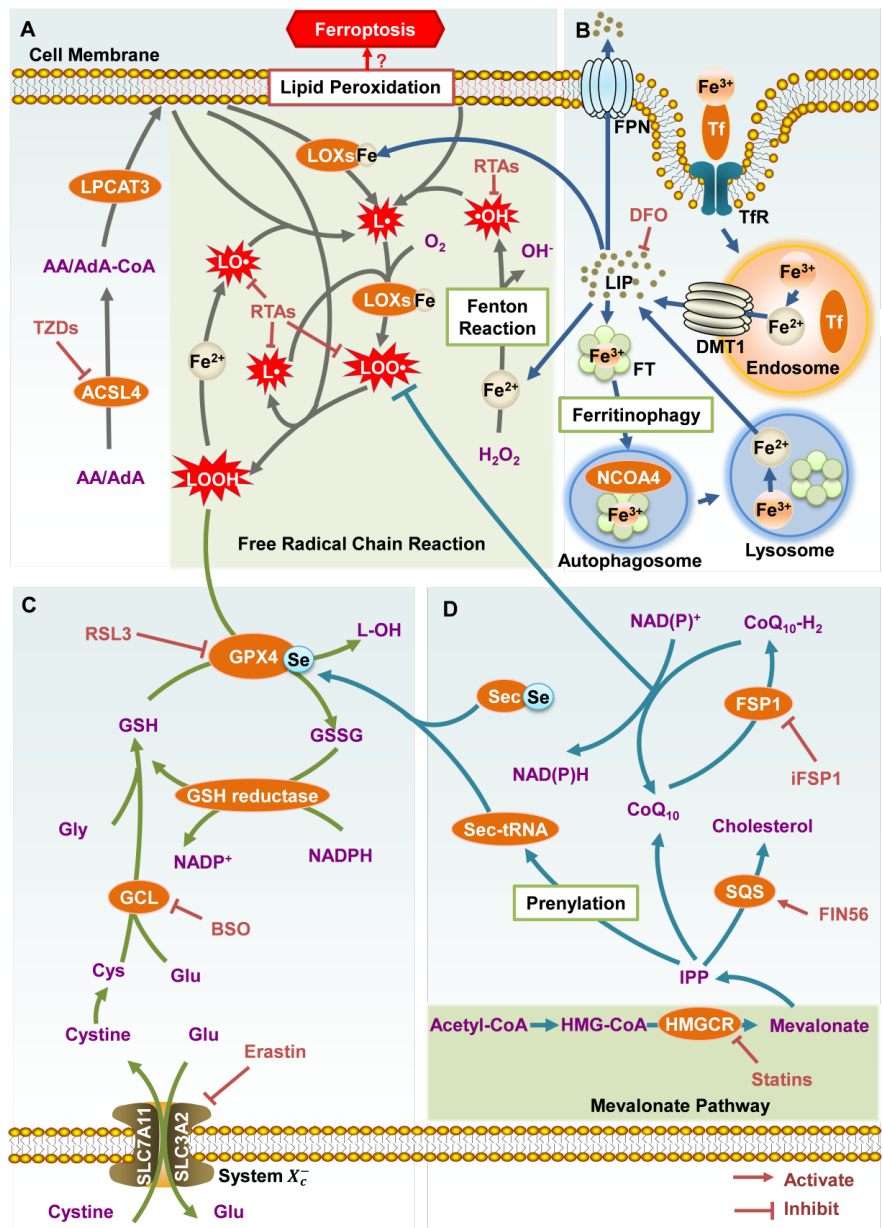
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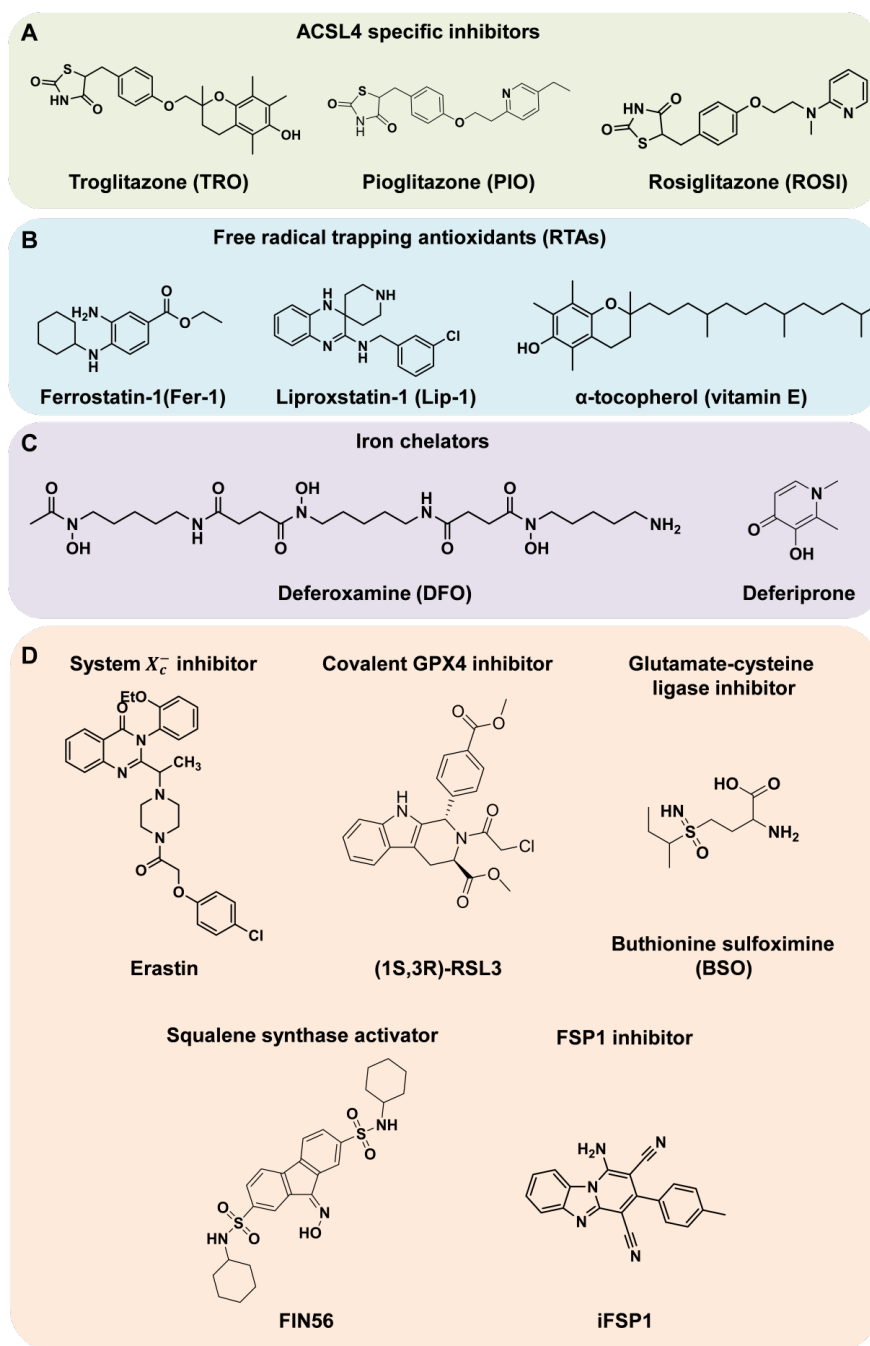
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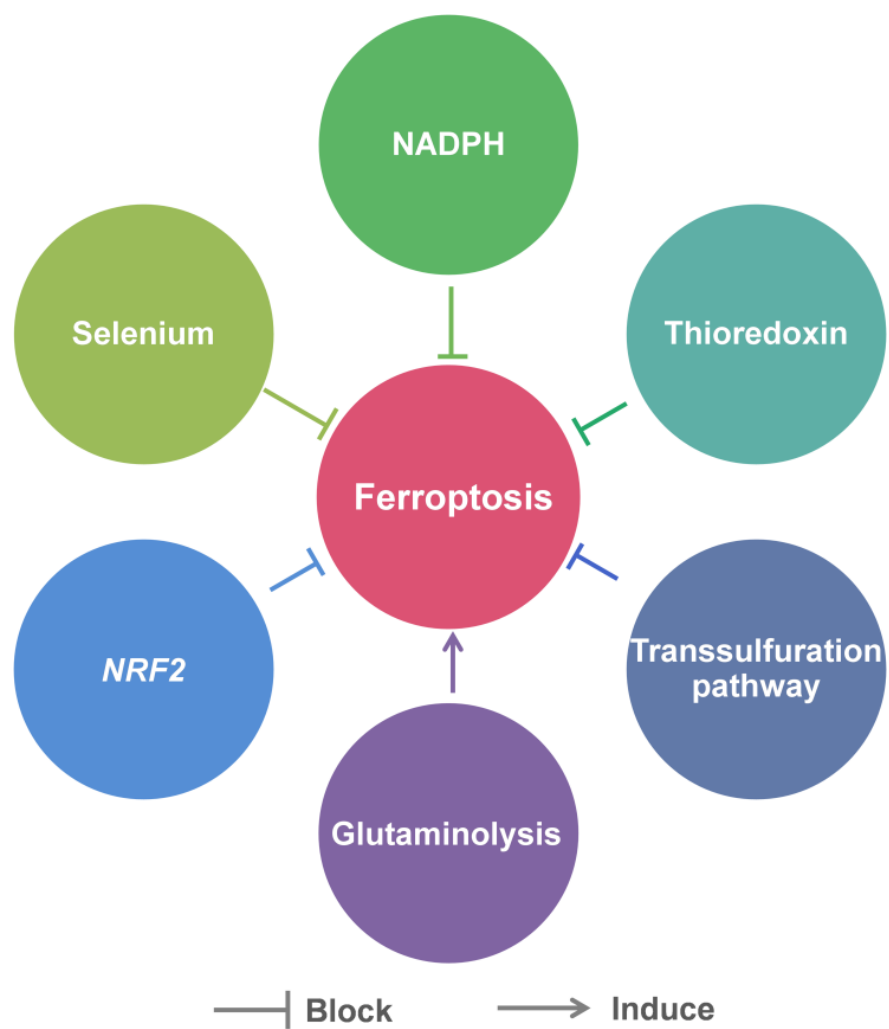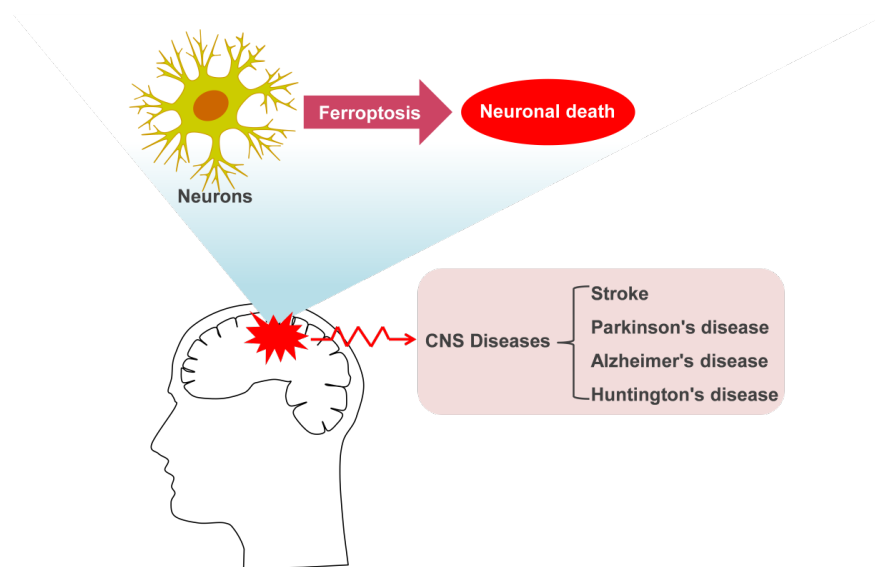
FIGURES

FIGURE 1 The key regulatory mechanisms of ferroptosis. (A) Lipid metabolism. (B) Iron metabolism. (C) Glutathione-dependent pathway. (D) CoQ$_{10}$-dependent pathway

A ACSL4 specific inhibitors

Troglitazone (TRO) **Pioglitazone (PIO)** **Rosiglitazone (ROSI)**

B Free radical trapping antioxidants (RTAs)

Ferrostatin-1(Fer-1) **Liproxstatin-1 (Lip-1)** **α-tocopherol (vitamin E)**

C Iron chelators

Deferoxamine (DFO) **Deferiprone**

D System X_c^- inhibitor Covalent GPX4 inhibitor Glutamate-cysteine ligase inhibitor

Erastin **(1S,3R)-RSL3** **Buthionine sulfoximine (BSO)**

Squalene synthase activator FSP1 inhibitor

FIN56 **iFSP1**

FIGURE 2 Regulators of ferroptosis. (A-C) Inhibitors of ferroptosis. (D) Inducers of ferroptosis

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FIGURE 3 Other factors that regulate ferroptosis

FIGURE 4 The relationship between ferroptosis and CNS diseases

TABLE

TABLE 1 Reagents that modulate ferroptosis in animal models or patients of CNS diseases

CNS Diseases	Reagents	Functions
Ischemic stroke	Extract of Naotaifang (Lan et al., 2020)	Regulating TFR1/DMT1 and SCL7A11/GPX4 pathways
	Carvacrol (Guan et al., 2019)	Increasing the expression of GPX4
	Tat-linked SelP Peptide (Alim et al., 2019)	Upregulating the expression of GPX4
Intracerebral hemorrhage stroke	DFO (Okauchi et al., 2010)	Chelating iron
	Fer-1 (Li et al., 2017)	Trapping free radicals
Parkinson's disease	Ferric ammonium citrate (FAC) (Zhang et al., 2020)	Upgrading ferritin levels
	Fer-1 (Do Van et al., 2016)	Trapping free radicals
	Deferiprone (Martin-Bastida et al., 2017)	Chelating iron
Alzheimer's disease	Lip-1 (Hambright et al., 2017)	Trapping free radicals
	Deferiprone (Rao et al., 2020)	Chelating iron
Huntington's disease	DFO (Chen et al., 2013)	Chelating iron
	Fer-1 and its analogues (Skouta et al., 2014)	Trapping free radicals