Review Article

Regulatory mechanism of ferroptosis, a new mode of cell death

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Abstract

Ferroptosis is a newly discovered process of cell death that differs from apoptosis, autophagy, and pyroptosis. It is closely related to tumor formation, diseases that damage tissue, and neurodegenerative diseases. Activation of the extracellular regulated protein kinase (EPK) pathway and acylCOA synthetase long-chain family member 4 (ACSL4) are indicative of ferroptosis. During ferroptosis, the mitochondrial volume becomes smaller and the double membrane density increases. The process of ferroptosis involves disruption of the material redox reaction, and changes in the levels of cystine, glutathione, NADPH, and increase of GPX4, NOX, and ROS. Iron increases significantly in ferroptosis. Divalent iron ions can greatly promote lipid oxidation, ROS accumulation, and thus promote ferroptosis. The occurrence and progress of ferroptosis are influenced by multiple factors and signaling pathways.

Keywords: Ferroptosis, Iron; Lipid, Active oxygen, Inhibitor, Inducer

INTRODUCTION

Cell death is a vital process. Cell death modes include programmed death, non-programmed death, and necrosis. In 2012, Dixon *et al* [1] described ferroptosis as a new mode of non-apoptotic cell death. Observed during study of the mechanism of erastin to kill human fibrosarcoma cells carrying the mutant oncogene RAS, ferroptosis is a mode of non-apoptotic cell death in which cells are injured by iron oxidation [2]. Ferroptosis occurs without typical characteristic of apoptosis, such as caspase activation. Instead, ferroptosis is related to the increase of iron within cells [3]. Ferroptosis is significantly different from autophagy, apoptosis, and pyroptosis both biochemically and morphologically (Table 1) [3]. Recent research has found that ferroptosis is closely associated with many diseases, such as Parkinson's disease (PD) [4], periventricular leukomalacia (PVL) [5], cerebral trauma [6], head and neck cancer (HANC) [7], hepatic carcinoma [8], and esophageal carcinoma [9]. Thus, targeting ferroptosis possibly provides a novel idea for treatment of these diseases.

Characteristics of ferroptosis

The manifestations of ferroptosis include changes of mitochondria, disruption of redox reaction equilibrium, accumulation of iron, and the build-up of active oxygen. These

accumulated substances can attack biomacromolecules and cause cell death [3].

The redox reaction of intracellular lipid oxide requires glutathione (GSH) and glutathione peroxidase 4 (GPX4), and the amount of lipid oxide would increase with a decrease in the content of intracellular GSH and decreased GPX4 activity. Instead, Fe^{2+} oxidizes the lipid in a reaction similar to the Fenton reaction thus producing a large number of reactive oxygen species (ROS) and causing ferroptosis to cells [10]. The long-chain acetyl coenzyme A and the synthetase family 4 (acylCOA synthetase long-chain family member 4, ACSL4) are sensitive monitoring indexes of ferroptosis and influencing factors in ferroptosis [11]. In metabolic disorders of intracellular lipid oxide, there can be production of a large number of lipids and lipid metabolites due to the catalytic action of iron ions [3]. At the morphological level, the volume of mitochondria in cells with ferroptosis is decreased and the bilayer of mitochondria is thicker and denser compared to normal cells. Additionally, the crista in the mitochondria decrease or even disappear [1,2]. Inflammatory responses also occur during ferroptosis [12] Overall but these are significantly different from that in cell apoptosis, autophagy, and pyroptosis (Table 1).

Regulatory mechanism of ferroptosis

The occurrence of ferroptosis is influenced by various factors and regulated by multiple signal pathways. Activation of the extracellular regulated protein kinases (ERK) pathway is considered as an important indicator of ferroptosis, and the extent of ferroptosis is directly proportional to the amount of ERK-phosphoric acid [13]. The regulation of cellular ferroptosis is illustrated in Figure 1 and further discussed.

Changes of mitochondria

In cells undergoing ferroptosis, the mitochondria show significant changes including decreases in volume and bilayer density. Erastin is an antineoplastic drug that can bind to the voltage-dependent anion channels (VDACs) of the mitochondrial outer membrane. This can cause VDAC2/3 to close, indirectly inducing the production of ROS, and promoting ferroptosis [13,14]. Dixon *et al* observed that the build-up of

Table 1: Characteristics of ferroptosis, apoptosis, autophagy and pyroptosis

| Type	Biochemical characteristics	Morphological characteristics	Activation conditions	Key factors
Ferroptosis	Lipid oxide and iron accumulation, decreased GSH, decreased activity of GPX4, high levels of ROS, activation of EPK pathway.	Smaller mitochondrial and thicker bilayer membrane with increased density, cristae decreased or eliminated inflammatory reactions, normal nucleus	Erastin, Sorafenib and RSL3	GPX4,TFR1 and SLC7A11
Apoptosis	DNA degradation, transcription stopped	Cell shrinkage, cell volume decreased, decreased space between organelles but no damage, nuclear condensation, chromatin accumulation, marginalization, formation of apoptotic bodies	Cell senescence, injury, infection, other pathological conditions	Caspase-3,Bcl-2 and Bax
Autophagy	Increased lysosomal activity and protein damage	Formation of autophagosomes, macroautophagy, microautophagy, and chaperone mediated autophagy	Lack of nutrition and oxidative stimulation	Beclin 1, ATG5 and ATG7
Pyroptosis	caspase-1 activates and releases inflammatory molecules	Cell expansion, damage and small holes appear in the cell membrane, allowing the release of cell contents. DNA breaks but no DNA ladder bands appear	Inflammatory reaction	Caspase-1 Caspase-11, NLPR1, NLPR3 NLRC4/NAIP,AIM2, IL-1β,IL-18
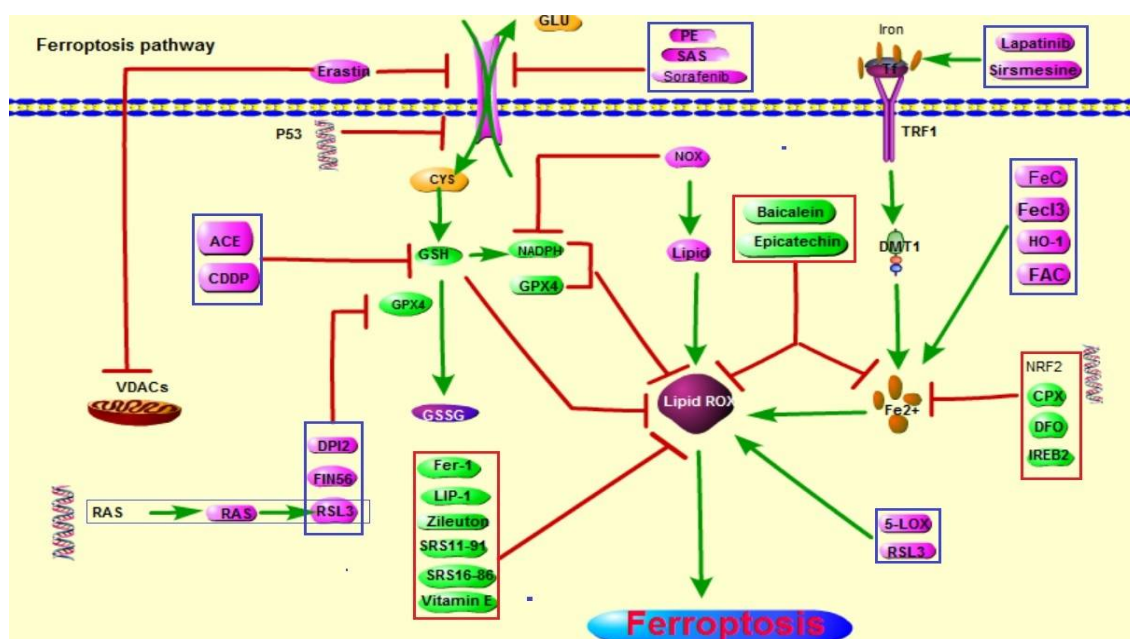
Figure 1: Signal pathway of ferroptosis, a cellular death pathway. Ferroptosis is dependent on iron lipid oxidation damage, which is mainly related to iron metabolism and regulation of intracellular redox reaction. Under some conditions, XC is inhibited and GSH and NADPH content are decreased, resulting in decreased intracellular antioxidant capacity. In the presence of iron ions, the lipid oxidation process is greatly enhanced, leading to the accumulation of lipid reactive oxygen species. Ultimately, increased lipid reactive oxygen species attack biological macromolecules, causing ferroptosis in cells

active oxygen may lead to mitochondrial damage. In addition, the increase in active oxygen affects calcium ions and the mediation of stress responses of the endoplasmic reticulum [15]. The link of active oxygen changes is critical to study the changes of mitochondria during ferroptosis, the downstream signals of ferroptosis, the regulation pathway of the ferroptosis signal, and the relationship between ferroptosis and endoplasmic reticulum responses. Additionally, recent work provides insight into the mutual metabolism between ferroptosis and other regulatory types of death [16,17].

Regulation of redox reactions

The process of ferroptosis involves disorders of redox reactions, decreases in the levels of cystine, glutathione, and NADPH, and increases in the levels of GPX4, NOXS, and ROS.

The cystine-glutamate antiporter (XC) on the cell surface is part of an important antioxidation system. The small molecule erastin can initiate ferroptotic cell death by binding and inhibiting VDAC2/3 (described above), and functionally inhibiting the XC cystine-glutamate antiporter system. Thus, cells that are treated with erastin

have decreased amounts of cysteine [1]. Because cysteine, glycine, and glutamic acid are required for glutathione synthesis [18], cells treated with erastin are unable to synthesize the antioxidant glutathione. The main role of Glutathione peroxidases (GPX) is to protect against oxidative damage. To do this, glutathione peroxidase acts to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water, preventing damage due to oxidation.

With decreased glutathione, there is less antioxidation activity, and the depletion of glutathione leads to excessive lipid peroxidation and cell death. Increased lipid oxidation and ROS promotes ferroptosis under the action of divalent ferrous ions. Related to this pathway, the P53 protein can also inhibit activity of XC-subunit-SLC711, resulting in decreased intracellular cystine and glutathione, increased ROS, and induction of ferroptosis [19].

NADPH is another important factor affecting the oxidation state. A decrease in the NADPH level promotes the accumulation of ROS and accelerates ferroptosis. Thus, the NADPH level also serves as an indicator of the sensitivity of cells to ferroptosis [20]. NADPH can be

synthesized via the pentose phosphate pathway, which is inhibited by H_2O_2. Intracellular glutathione can act to counter these inhibitory effects of H_2O_2. Thus, a decrease in the level of glutathione can result in decreased NADPH level. NADPH and GSSG react to generate GSH, and the occurrence of this reaction is decreased when the content of NADPH decreases [20].

The RAS gene is an oncogene, and expressed RAS induces ferroptosis. RAS can promote the synthesis of RSL3, a class of cell-lethal chemical compounds that promotes cell death. In addition, RAS gene expression promotes the generation of NAPDH oxidase (NOX). NOX acts to decrease the content of NADPH and promote the accumulation of ROS and cell ferroptosis [1]. When the activity of the glutathione oxidase GPX4 is up-regulated, it can inhibit RAS activity. When RAS is inhibited, it is not available to promote RSL3 synthesis, so the RSL3 level decreases, resulting in less cell death. When RSL3 is up-regulated, RSL3 can bind to GPX4 and deactivate it, inhibiting the synthesis of glutamine and promoting the accumulation of ROS [21]. Glutamine is decomposed into glutamic acid, aspartic acid, and alanine, which all participate in the tricarboxylic acid reaction, and are closely associated with carcinogenesis [22]. Glutamic acid also participates in the synthesis of glutathione. In addition, glutamine can be decomposed into L-glutamine [23], which can promote the generation of ROS. Thus, control of glutamine metabolism can achieve ferroptosis occurrence [24].

Regulation of iron metabolic reactions

Iron has many important functions within the human body, such as the synthesis of hemoglobin, participation in oxygen transport, participation in synthesis of ATP, as the cofactor of some proteins in the electron transport chain of mitochondria, and participation in the synthesis of DNA as the ribonucleotide reductase [25]. The content of iron increases significantly during ferroptosis. Divalent iron ions can significantly promote lipid oxidation, the accumulation of ROS, and ferroptosis.

When ferric ions cannot bind to proteins or other ligands in an appropriate manner, the H_2O_2-dependent Fenton reaction can form ROS with metabolic toxicity. ROS attacks biomacromolecules, thus leading to cell death. Ferritin and its related gene-ferritin light chain (FTL) and ferritin heavy chain 1 (FTH1) can regulate the storage of ferric ions. The heat shock protein B1 (HSPB1) decreases the concentration of intracellular ferric ions by inhibiting the expression of TFR1. Thus, over-expressed HSPB1 can inhibit ferroptosis [26]. Inhibiting the major transcription factor for iron metabolism iron response element binding protein 2 (IREB2) can significantly increase the expression of FTL and FTH1 and inhibit ferroptosis that is induced by erastin [27]. The iron regulator protein (IRP) is a trans-acting factor that can promote the effect of erastin to cause ferroptosis. Heme oxygenase-1 (HO-1) also plays an important role in ferroptosis [3]. The intracellular HO-1 can degrade heme into iron and porphyrin, thus increasing the killing effect of erastin. Overall, the catabolism of hemachrome can promote ferroptosis. The HO-1 inhibitor, zinc protoporphyrin ZnPP, can inhibit ferroptosis induced by erastin. Nuclear receptor coactivator 4 (NCOA4) is a selective carrier receptor for ferroptosis, promoting the degradation of ferritin through autophagy. Over-expressed NCOA4 can increase the degradation of ferritin and promote ferroptosis [28]. Thus, the targeted regulation of ferritin autophagy can regulate iron metabolism in cells, cause iron overloading, and lead to cell injury.

Modulators of ferroptosis

The development and progression of ferroptosis can be influenced by many factors and multiple signal pathways. Modifiers of ferroptosis will be described in the following paragraphs, including iron element inhibitors, lipid peroxidation inhibitors, iron element inducers, lipid peroxidation inducers, and other modifiers.

Iron inhibitors

Iron inhibitors include iron chelators such as deferoxamine (DFO), deferoxamine mesylate, 2,2'–bipyridine and others. These iron chelators can effectively inhibit ferroptosis [1]. Additionally, the nuclear factor E2-related factor 2 (NRF2) inhibits ferroptosis by degrading the iron taken in by cells and limiting the production of ROS.

Lipid peroxidating inhibitors

Lipid peroxidating inhibitors include fertostain-1, liproxstain-1, zileuton, and 5-LOX. Fer-1 is an antioxidant containing arylamine, which can inhibit ferroptosis by inhibiting lipid peroxidation [3]. The first and second derivatives of Fer-1 (SRS11-92 and SRS16-86, respectively) exhibit more stable inhibitory activities, resulting in increased tissue damage resistance is strengthened significantly [29]. Liproxstain-1 includes an amide and a sulfamide subunit. Stable and well adsorbed, the drug can significantly inhibit ferroptosis without interfering

with other cell death modes [30]. The 5-lipoxygenase (5-LOX) inhibitor zileuton can inhibit the generation of cellular active oxygen and further inhibit glutamic acid toxicity and cellular ferroptosis, thus exerting neuroprotective effects [31]. The NOX inhibitor dimethylaniline can partially inhibit erastin-induced ferroptosis in cells [1]. Other experiments showed that butylated hydroxytoluene, TROLOX, ebselen, α-tocopherol, vitamin E [32], lysosome activity inhibitors such as lostoxin A, pepstatin methyl ester, and ammonium chloride (NH4CL) [33] inhibit ferroptosis by inhibiting lipid peroxidation.

Iron metabolism inducers

Ferroptosis is related to the increase of iron within cells. Iron metabolism inducers can also induce ferroptosis. Sirsmesine or lapatinib can raise the level of ferric chloride (Fecl3) within the cells and induce ferroptosis by regulating the expression of transferrin in iron metabolism. Sirsmesine and lapatinib can treat breast cancer [34]. The co-incubation with cells of other iron metabolism inducers, such as ammonium ferric citrate, ferric citrate, or ferric chloride can increase the rate of ROS generation and the cell mortality rate [1].

Regulators of oxidative stress

Erastin, heme oxygenase-1 (HO-1), erastin derivatives such as piperazine erastin (PE) [22] and imidazoline erastin [22] are all regulators of oxidative stress and are likely to induce ferroptosis as they have high solubility and stability in the human body. Sorafenib can induce ferroptosis and increase the toxicity of hepatoma carcinoma cells thus promoting tumor decline [35]. NAPDH oxidase (NOX) acts to produce ROS, and 5-lipoxygenase (5-LOX) also can promote generation of lipid ROS [36] to induce ferroptosis of cells.

Other types of regulators

Because of the double function of regulating lipid peroxidation and iron metabolism, baicalein has better regulatory effects than deferoxamine ferroptosis inhibitors such as Fer-1 and deferoxamine mesylate, suggesting a good potential for use as therapeutic drugs [37]. Epicatechin regulates lipid ROS metabolism for neuroprotection by regulating nuclear respiratory factors [38] and can also inhibit the ferroptosis pathway for neuroprotection by inhibiting ferroptosis-related genes (Gs, Rpl8, and ATP5G3).

Other regulators such as sulfasalazine (SAS) inhibit cystine adsorption via the cystine/glutamic acid transporters, thus disrupting the redox equilibrium within cells and causing oxidation death dependent on ferric ions [1]. Cisplatin (CCDP) can increase the level of active oxygen within the cells, induce ferroptosis and specific morphological changes and chemical changes in tumor cells, thus inhibiting the growth of tumor cells. Meanwhile, the activity of CDDP can be partially reversed by Fer-1, suggesting that CDDP can induce ferroptosis [39]. As many cancers develop to advanced stages, cancer cells have developed resistance to chemotherapeutics, resulting in the failure of chemotherapy, allowing the tumor to grow and spread further. 1, 2-dioxolane compound (FI-NO2), a peroxide with five rings that can not only stably and effectively induce ferroptosis of cells in a high-temperature environment but also overcome some common drug resistance. This suggests that it will exert perfect efficacy in cancer treatment [40].

Ferroptosis-related diseases

Tumors

Ferroptosis is closely associated with the occurrence, progression, and treatment of many tumors. Sorafenib is often used for the treatment of advanced liver cancer, and the state of the Rb protein is an important parameter indicating ferroptosis of liver cancer cells induced by this drug. A decrease in the level of Rb protein may promote ferroptosis in cells [8]. Knock-out of nuclear factor E2-related factor 2 (NRF2) and its signaling pathway downstream target genes can strengthen the activity of sorafenib and inhibit proliferation of liver cancer cells. Acetaminophen can induce ferroptosis in primary hepatic carcinoma cells, and Ferrostatin-1 can inhibit the induction role of acetaminophen [40]. The NOX inhibitor dimethylaniline partially inhibits the induction of ferroptosis by erastin in human lung cancer. Additionally, glucose-6-phosphate dehydrogenase and phosphoglycerate acid dehydrogenase can also protect human lung cancer cells against erastin-induced ferroptosis when the pentose phosphate pathway is knocked out. Pancreatic cancer cells are highly resistant against apoptosis. Using artesunate for treatment of pancreatic cancer can activate ferroptosis in cancer cells [4]. In breast cancer, down-regulation of the MUCI-C/System Xc-signal pathway can induce ferroptosis in triple-negative breast cancer (TNBCA) cells, killing the cancer cells or weakening the cell self-renewal and tumorigenicity [41]. In addition, ferroptosis can occur in renal cancer [8], esophageal carcinoma

[9], lymphoma [42], head and neck cancer [7], glioma [43], and rhabdomyosarcoma [44].

Tissue damage

Ferroptosis inhibitor, including iron inhibitor and lipid peroxidating inhibitor can effectively repairs ischemia reperfusion injury (IRI). Tissue damage triggered by ischemia reperfusion, such as myocardial damage, can be treated by inhibiting ferroptosis [24]. The ferroptosis inhibitor SRA16-86 can be used to treat renal injury induced by ischemia reperfusion and oxalic acid crystals, rhabdomyolysis, and acute renal failure (ARF) [45]. The ferroptosis inhibitor liproxstain-1 can completely block lipid peroxidation and improve hepatic injury in mice caused by ischemia reperfusion [10].

Neurogenic diseases

The accumulation of iron in the brain and the generation of ROS promote craniocerebral injury [46]. Hypoxia inducible factor-1 (HIF-1) can regulate the microenvironment under a hypoxia environment through multiple vascular endothelial growth factors and signal pathways. HIF-1 can also reduce the expression of HIF-1 to exacerbate cerebral ischemia. Hypoxia inducible factor-1 prolyl-hydroxylase (HIF PHD) can decompose HIF [47,48]. The death of developing oligodendrocytes causes periventricular leukomalacia, and is dependent on iron and is increased by an increase in lipid ROS [49]. In the peripheral nervous system, knockout of GPX4 can cause lipid peroxidation and mitochondrial dysfunction, thus accelerating ferroptosis of neurons and causing paralysis of mice, but only minor effects on cortical neurons of adult rats [12]. The iron chelators, Fer-1, and PKC inhibitor can significantly inhibit the signal pathways of ferroptosis in neurodegenerative diseases [4]. Ferroptosis also plays important roles in various other nervous system diseases such as Huntington's (HD), Alzheimer's disease (AD), motor nerve degeneration, and paralysis [4].

Other diseases

Wu *et al* found that ferroptosis participates in the death of keratinocytes due to the loss of GSH. A high dose of vitamin E can inhibit ferroptosis of skin keratinocytes and reduce skin injury [50]. Mai found that the loss of GPX4 in T cells resulted in accelerated accumulation of lipid peroxide and induction of ferroptosis. Inhibition of ferroptosis can promote the survival and proliferation of T cells and protect immunological functions [32].

FINAL REMARKS

Research on ferroptosis is in the preliminary stage and mechanistic details remain to be characterized [1]. The evaluation of ferroptosis primarily is based on morphological changes in cells and the accumulation of ROS. Similar to the activity of caspases in apoptosis, identification of an appropriate marker for ferroptosis will be highly useful [2]. Studies of ferroptosis mechanism have focused on oxidative damage and the iron metabolism signal pathway but additional topics are also worthy of study. For example, many triggers of ferroptosis have been reported in different tissues, but the main signal pathway in ferroptosis remains unclear [3]. Iron regulation and control of oxidation are complex processes that require further analysis [4]. The establishment and optimization of a ferroptosis research model is also an important requirement for more advanced research on ferroptosis [5]. Additionally, it is important to better understand the association of ferroptosis and different diseases to better determine the kind of drugs that might be most effective for disease treatment. Overall, increased study of ferroptosis should facilitate strategies to target this cell death mode as a treatment for many diseases.

DECLARATIONS

Acknowledgement

Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Da Liu, Guoya Li, Ang Jia, Yanyan Zhao, Chenxi Cao reviewed the literature and wrote the manuscript. Xuekun Xing conceived the idea, reviewed the literature, and edited the manuscript.

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