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Ferroptosis and its emerging roles in acute pancreatitis

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Abstract

Acute pancreatitis (AP) is a common and potentially life-threatening pancreatic inflammatory disease. Although it is usually self-limiting, up to 20% of patients will develop into severe AP. It may lead to systemic inflammatory response syndrome and multiple organ dysfunction, affecting the lungs, kidneys, liver, heart, etc. Surviving patients usually have sequelae of varying degrees, such as chronic hyperglycemia after AP (CHAP), pancreatic exocrine insufficiency, and chronic pancreatitis. Lacking specific target treatments is the main reason for high mortality and morbidity, which means that more research on the pathogenesis of AP is needed. Ferroptosis is a newly discovered regulated cell death (RCD), originally described in cancer cells, involving the accumulation of iron and the depletion of plasma membrane polyunsaturated fatty acids, and a caspase-independent RCD. It is closely related to neurological diseases, myocardial infarction, ischemia/reperfusion injury, cancer, etc. Research in the past years has also found the effects of ferroptosis in AP, pancreatic cancer, and AP complications, such as acute lung injury and acute kidney injury. This article reviews the research progress of ferroptosis and its association with the pathophysiological mechanisms of AP, trying to provide new insight into the pathogenesis and treatment of AP, facilitating the development of better-targeted drugs.

Keywords: Acute pancreatitis; Ferroptosis; RCD; Autophagy; GPX4; PUFAs; Regulated cell death

Introduction

Acute pancreatitis (AP) is an inflammatory disease. Gallstones are the most common cause of AP (approximately 40%–50%). In most countries, alcohol is the second leading cause of AP, accounting for 20%. Less common causes include drugs, endoscopic retrograde cholangiopancreatography, hypercalcemia, hypertriglyceridemia, infection, genetics, autoimmune diseases, and (surgical) trauma.[1] The exact pathophysiological mechanism of AP is still a mystery, but in the past 10 years, we have made great progress in understanding the pathophysiological mechanisms of AP. Studies have elucidated the mechanisms of calcium-mediated acinar cell injury and death and the importance of store-operated calcium entry channels and mitochondrial permeability transition pores. The cytoprotective role of the unfolded protein response and autophagy in preventing sustained endoplasmic reticulum stress, apoptosis, and necrosis has also been characterized, as has the central role of unsaturated fatty acids (UFAs) in causing pancreatic organ failure. The study also sheds light on the central role of UFAs in causing pancreatic organ failure.[2] In recent years, various regulatory cell deaths (RCDs), such as apoptosis, pyroptosis, autophagy, and necroptosis, have also been found to play important roles in the pathogenesis of AP. The progression of AP is closely related to the regulatory transitions between different RCDs. Ferroptosis is a rising star, and studies have shown that inhibition of ferroptosis can alleviate reactive oxidative stress (ROS) and inflammation during AP and has a beneficial effect on multiple organ failure, such as acute kidney injury (AKI), induced by severe AP (SAP). This result highlights the close relationship between ferroptosis and AP.[2-4]

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Background of Ferroptosis

Ferroptosis is a new RCD term coined in 2012 by Dr. Brent R Stockwell's laboratory.[5] Researchers at the time found in experiments that the cell death induced by erastin and RAS-selective lethal 3 (RSL3) was not affected by caspase inhibitors (Z-VAD-FMK), necrostatin-1, and autophagy inhibitors (chloroquine, 3-methyl, and adenine). In 2018, the Nomenclature Committee for Cell Death officially defined ferroptosis as "a form of RCD initiated by oxidative perturbations of the intracellular microenvironment that is under constitutive control by glutathione peroxidase 4 (GPX4) and can be inhibited by iron chelators and lipophilic antioxidants".[5,6] When ferroptosis occurs, the cells exhibit necrotic-like changes. These features include loss of plasma membrane integrity, cytoplasmic organelle swelling, and moderate chromatin condensation. It is worth noting that, according to reports,

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ferroptosis occurring in one cell can spread to neighboring cells in a fast propagating wave, which may cause extensive tissue damage. Occasionally, ferroptosis is accompanied by cell shedding and aggregation and an increase in autophagosomes.[7,8] From the microscopic point of view, the mitochondria of ferroptotic cells exhibit shrinkage, an electronically dense ultrastructure, cristae reduction/disappearance, and even outer mitochondrial membrane rupture.[6] Molecular dynamics also show that an increase in lipid peroxidation directly increases the permeability of the membrane, changes the shape and curvature of the membrane, promotes the availability of oxidants, and ultimately leads to cell death.[9] With the deepening of research on ferroptosis, the role of ferroptosis and related regulatory effects has gradually attracted the attention of researchers. As the most extensive and oldest method of cell death, it brings together iron, selenium, amino acids, lipids, and redox chemistry, previously dispersed elements of cell metabolism, into a tight network and maintains homeostasis by participating in various pathways.[7]

Key factors of ferroptosis

GPX4

GPX4 is a key synergistic factor of ferroptosis. It can convert glutathione (GSH) into oxidized glutathione (GSSG) and convert cytotoxic lipid peroxide (PLOOH) into corresponding alcohol (PLOH), ultimately reducing the accumulation of ROS [Figure 1]. In this process, reduced nicotinamide adenine dinucleotide phosphate (NADPH) acts as an electron donor. NADPH abundance can be used as a predictor for the outcome of a ferroptotic event. At the same time, the level of cytosolic NADPH can be controlled by Metazoan SpoT Homolog 1 to control ferroptosis.[10-12] There are three subtypes of GPX4: mitochondria, cytoplasm, and nucleus, but which subtype is the main regulator of the antiferroptotic is unclear. In addition to ferroptosis, GPX4 also inhibits cell apoptosis, necroptosis, and pyrolysis, suggesting that lipid peroxidation may be a common signal that induces different types of RCD.[13-15] GPX4 can be inhibited by direct inhibitors such as RLS3 or

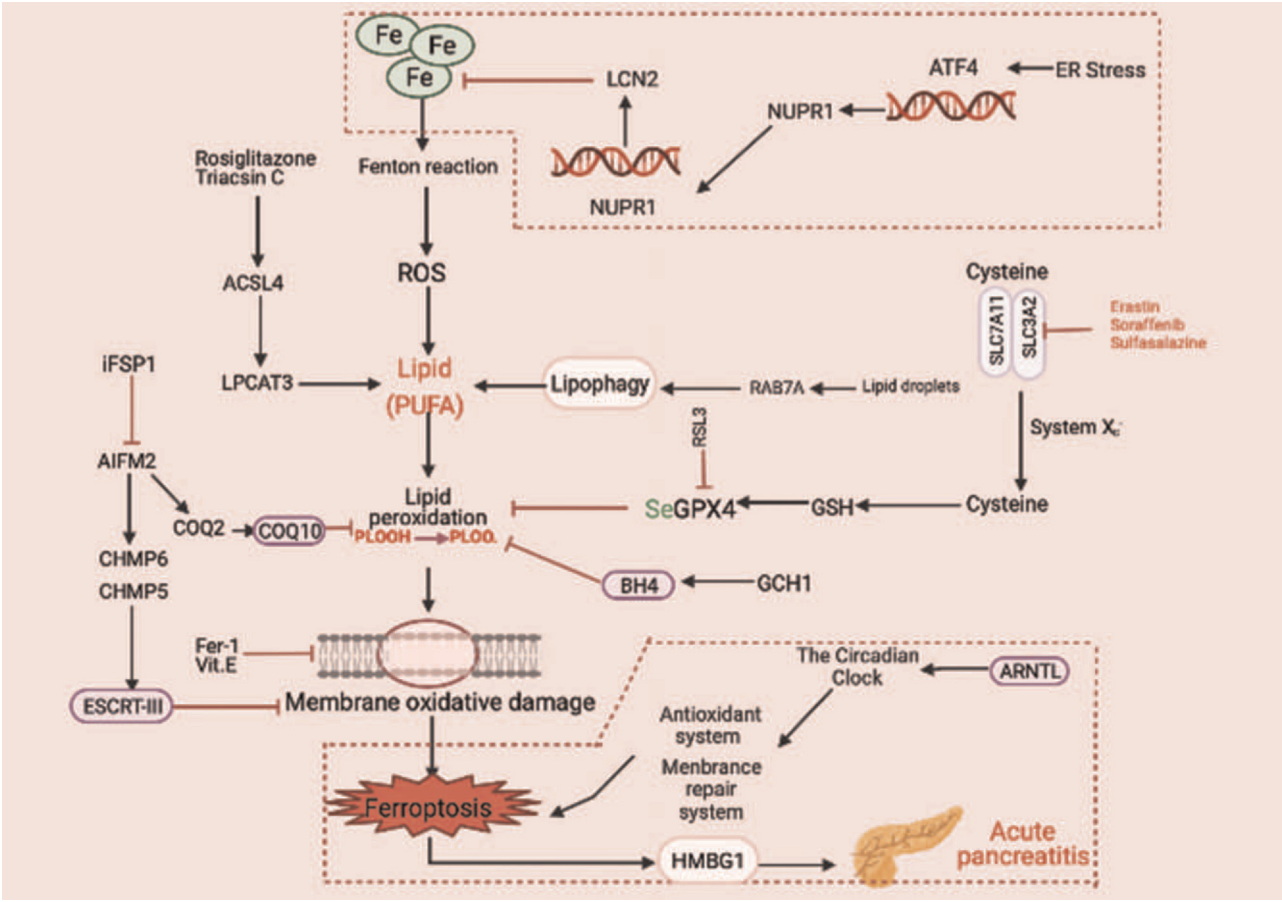

Figure 1: Schematic description of the signaling pathway of ferroptosis. Ferroptosis is a form of cell death that relies on the balance between iron accumulation-induced ROS production and the antioxidant system during lipid peroxidation. The picture contains three currently known pathways: Lipid Oxidation Metabolism, GSH Metabolic Pathway, Iron Metabolic Pathway, and some related mechanisms of AP and ferroptosis. Several pharmacological inducers have been shown to induce ferroptosis (eg, erastin and RSL3). A variety of ferroptosis inhibitors inhibit iron death from various pathways (eg, Fer-1, lip-1, BP, and DFO). The red box shows the latest research on AP and ferroptosis.[3,4,8,19,32,43] AP: Acute pancreatitis; DFO: Deferoxamin; GSH: Glutathione; LIP: Labile iron pool; RSL3: RAS-selective lethal 3; ROS: Reactive oxidative stress; ACSL4: Acyl-CoA synthetase long chain family member 4; LPCAT3: Lysophosphatidylcholine acyltransferase 3; ER Stress: Endoplasmic reticulum stress; ATF4: Activating transcription Factor 4; GPX4: Glutathione peroxidase 4; NUPR1: Nuclear protein 1 transcriptional regulator; LCN2: Lipocalin 2; PUFA: Polyunsaturated fatty acid; ESCRT-III: Endosomal sorting complexes required for transport; SLC7A11: Solute carrier family 7 member 11; SLC3A2: Solute carrier family 3 member 2; ARNTL: Aryl hydrocarbon receptor nuclear translocator-like; ATF4: Activated transcription factor 4; Fer-1: Ferrostatin-1; CHMP6: Charged multivesicular body protein 6; CHMP5: Charged multivesicular body protein 5; GCH1: GTP cyclohydrolase 1; BH4: Metabolite tetrahydrobiopterin 4; HMGB1: High mobility group box 1; FSP1: Ferroptosis suppressor protein 1; AIFM2: Apoptosis-inducing factor 2; COQ10: Coenzyme 10; COQ2: Coenzyme 2.

by the consumption of cellular GSH. Conversely, ferroptosis can be prevented by inhibiting lipid peroxidation.[13]

Arachidonate lipoxygenases (ALOXs)

ALOXs are iron-containing lipid dioxygenases that catalyze the insertion of oxygen into polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA) and linoleic acid.[10] Among the six members of the ALOX family in humans, ALOX5, ALOX12, arachidonate lipoxygenase 15 (ALOX15), ALOX15B, and ALOXE3 promote ferroptosis in a cell type-dependent manner. Therefore, the basal expression of ALOX family members may inform the sensitivity of cells to ferroptosis.[15,16] AlOX5-deficient mice exhibit a suppressed response to inflammation and reduced organ injury, including pancreas, lung, and liver injury, as well as amelioration of an Alzheimer's disease-like phenotype.[17]

Fatty acids

Fatty acids — including saturated fatty acids, monounsaturated fatty acids (MUFAs), and PUFAs — have a variety of functions in cells, as structural "building blocks" of cell membranes, energy providers, and even signal molecules. Interestingly, PUFAs (especially AA and adrenic acid) promote ferroptosis, whereas MUFAs (especially oleic acid and palmitoleic acid) limit ferroptosis because the structures of PUFAs and MUFAs differ in their susceptibility to lipid peroxidation.[9,18] The enzymes acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) participate in the incorporation of PUFAs into membranes.[10]

X_c^-

X_c^-, located at the most upstream region, is a cystine/glutamate antiporter that is composed of light chain units (Xct, SLC7A11, is a ferroptosis regulator that is highly expressed in human cancers and is associated with chemotherapy drug resistance) and heavy chain units (CD98 hc, SLC3A2) composed of disulfide bond heterodimers.[19] It is a sodium-independent antiporter that imports cystine and exports glutamate at a ratio of 1:1 in an ATP-dependent manner.[2] Inhibition of systemic X_c^- depletes intracellular cysteine, resulting in a decrease in GSH concentration, triggering oxidative stress, and increasing the sensitivity of cells to ferroptosis.[20] The expression or activity of SLC7A11 is regulated by many factors, such as TP, nuclear factor erythroid -related factor (Nrf2), BRCA1-related protein 1 (BAP1), mucin 1 (MUC1), and Beclin-1. In short, inhibiting the SLC7A11 pathway is one of the most critical upstream mechanisms that cause ferroptosis.[9,10]

Iron

Intracellular iron is subtly regulated to maintain iron homeostasis. Iron regulatory proteins (IRP1 and IRP2) modulate cellular Fe^{2+} concentrations, and a variety of proteins regulate import, storage, release, and export of iron. Most intracellular Fe^{2+} are stored in ferritin and iron-containing proteins, and the amount of free Fe^{2+}, also called the cellular labile iron pool (LIP), is very limited. In mammalian cells, a portion of the cellular iron can be distributed in mitochondria, cytosol, nucleus, and lysosomes. Although the amount is small, the cells are sensitive to iron concentration, and a small fluctuation in concentration can cause big reactions.[19] Iron chelators are strong inhibitors of ferroptosis, including deferoxamine (DFO), deferiprone, and ciclopirox. Two iron chelators, DFO, and deferiprone, are currently in phase II clinical trials for the treatment of neuronal damage in Parkinson's disease, dementia, intracranial hemorrhage, and ischemic stroke.[2]

Inducers and Inhibitors of Ferroptosis

Ferroptosis can be caused by either the external or the internal pathway. The external pathway is initiated by regulating the transporters (eg, inhibition of the amino acid antiporter system X_c^- or activation of the iron transporters transferrin and lactotransferrin), whereas the internal pathway is mainly through blocking the expression or activity of intracellular antioxidant enzymes, such as GPX4. In addition to small-molecule compounds and drugs, certain stresses (eg, high temperature, low temperature, hypoxia, and radiation) can also cause ferroptosis.[9,10]

Ferroptosis inducers can be roughly divided into three categories: (1) system X_c inhibitors; (2) inhibitors of GPX4; and (3) compounds that indirectly inhibit the activity of Gpx4 through GSH depletion.[11] Inhibitors of ferroptosis can be divided into lipophilic antioxidants (such as iron statin-1 and alpha-tocopherol), iron chelators (such as DFO), and deuterated polyunsaturated fatty acid phospholipids (PUFAs).[21]

Metabolism and Ferroptosis

Current studies have shown that there are three main aspects related to ferroptosis pathways: (1) the GSH/Gpx4 pathway, X_c^- inhibitory system, sulfur transfer pathway, and p53 regulatory axis; (2) regulation of iron metabolism by autophagy protein 5 and 7 (ATG5-ATG7) and nuclear receptor coactivator 4 (NCOA4) pathway and iron-responsive element-binding protein 2 are involved in ferritin metabolism, and the p62-Kelch-like ECH-associated protein 1 (Keap1)-Nrf2 regulatory pathways; and (3) lipid metabolism pathways, such as p53, ALOX15, ACSL4, and LPCAT3. Subsequent studies also found GTP cyclohydrolase 1-tetrahydrobiopterin 4 (GCH1-BH4), E-cadherin-NF2-Hippo-YAP, and NADPH-ferroptosis suppressor protein 1 (FSP1)-coenzyme Q10 (CoQ10) pathway.[18,22]

These Gpx4-dependent and Gpx4-independent regulatory pathways are closely connected to various metabolic pathways and subcellular organelles (such as mitochondria, ER, Golgi, nucleus, lysosomes, and peroxisomes) and play a role in cancer and ischemia-reperfusion injury (IRI) diseases[13] [Figure 1].

Ferroptosis and AP

Ferroptosis, as a new RCD first discovered in tumor cells, has a relatively clear relationship with tumors and IRI,

but there are few studies on its correlation with AP. The pathogenesis of AP is closely related to lipid peroxidation and ROS. At the same time, the pancreas is not only a digestive organ but also one of the main organs for iron storage, all of which are closely related to ferroptosis.[2-4] As it is beyond the scope of this review to cover all mechanisms of ferroptosis, we summarize only the potential redox mechanisms that regulate ferroptosis in AP.

Lipid oxidation metabolism

During ferroptosis, lipid oxidation occurs as an "intermediate event." Lipids are hydrocarbon-containing biomolecules that form the basis of cell membrane structure and function. During ferroptosis, PUFAs, especially AAs, are highly susceptible to peroxidation, thus causing the destruction of lipid bilayers and disrupting membrane function.[14,21] ACSL4 and LPCAT3 are two key enzymes required for the production of PUFAs. Lipid peroxidation mediated by the ALOX family generates toxic phospholipid hydroperoxides (PLOOHs).[13,23] The rupture of the fatty acid backbone can also lead to the formation of reactive aldehydes such as 4-hydroxy-nonenal and malondialdehyde (MDA), which can attack proteins or DNA, amplifying cellular damage.[16] MDA is derived from the decomposition of AAs and larger PUFAs through enzymatic and non-enzymatic pathways.[24] MDA overdose can cause many human diseases, such as Alzheimer's and Parkinson's diseases, cancer, cardiovascular diseases, and diabetes.[25] Lipid peroxidation affects all cell membranes. Specifically, it affects the lipid bilayer and the subcellular membranes of the mitochondria, ER, and lysosomes. The ER is the first site of lipid synthesis within the cell; thus, lipid peroxidation might occur here. Mitochondrial membranes may also be oxidized during ferroptosis, and the accumulation of LOOH at the mitochondrial membranes can increase permeability, thus explaining the swelling ofmitochondrial and the rupture of the outer membrane.[11,24,25]

During AP, stored triglycerides undergo lipolysis, and UFAs, such as linoleic, oleic, and linolenic acids, can inhibit mitochondrial complexes I and V, increase the levels of inflammatory mediators, such as tumor necrosis factor (TNF-α), CXC ligand (CXCL) 1, CXCL2, and other chemokines, enhance the inflammatory response, and cause cytotoxicity. This reaction releases toxic free fatty acids that can lead to further acinar cell injury and organ failure, and ultimately to further damage to acinar cells and organ failure, while converting mild AP to SAP.[2,26] At the same time, UFAs are polar and often combine with Ca^{2+}, leading to saponification and inactivation of lipid necrosis and hypocalcemia during SAP. Changes in Ca^{2+} can cause mitochondrial dysfunction and abnormal activation of pancreatic enzymes.[26,27] There is no direct evidence showing that the accumulation of UFAs in AP induces ferroptosis, but we found that conditional knockout of ARNTL disrupts the circadian rhythm ofthe pancreas and increases the susceptibility to pancreatitis associated with ferroptosis by inhibiting the expression of various antioxidants or membrane repair genes (eg, SLC7A11, GPX4, superoxide dismutase 1, thioredoxin, NFE2L2, and charged multivesicular body protein 5 [CHMP5]).[3,9,25] The transcription factor aryl hydrocarbon receptor nuclear translocator-like protein 1/brain and muscle ARNT-like 1 (ARNTL/BMAL1) is a central component of the mammalian circadian clock because it regulates the expression of other clock-controlled genes, such as those coding for the period circadian regulator and cryptochrome circadian regulator (CRY) families[28] [Figure 1].

GSH metabolism

Classic X_c^- GSH-Gpx4 pathway

Amino acid metabolism is closely related to ferroptosis. Glutamate and glutamine are important regulators of ferroptosis. The high concentration of extracellular glutamate inhibits system X_c^- and induces ferroptosis, which may explain the toxic effects of glutamate when it accumulates to high concentrations in the nervous system.[13] The X_c^- GSH-Gpx4 pathway is one of the most classic pathways in the study of ferroptosis.[19] GSH is a tripeptide containing cysteine and an important intracellular antioxidant. Its production mainly depends on the uptake of cystine mediated by amino acid antiporter system X_c^- and attendant reduction of cystine to cysteine or, alternatively, on the generation of cysteine through the transsulfuration pathway regulated by cysteinyl-transfer ribonucleic acid (RNA) synthetase 1.[10,22] Ferroptosis may be further regulated by an amino acid sensor: the mechanistic target of rapamycin kinase or an energy sensor: adenosine monophosphate-activated protein ki-nase. These findings emphasize the complexity and plasticity of ferroptosis regulation, which is strongly affected by the cellular context.[10,21] The Food and Drug Administration (FDA)-approved tyrosine kinase inhibitor sorafenib can trigger ferroptosis in distinct cellular models by depleting GSH upon system X_c^- inhibition, whereas altretamine (an FDA-approved alkylating agent) has been recently identified as a potential inhibitor of GPX4 by a regulatory network genome-wide system strategy. Therefore, the antineoplastic effects ofsorafenib and altretamine may partially stem from the activation of ferroptosis.[6]

Classic X_c^- GSH-Gpx4 pathway in AP

During AP, damaged pancreatic acinar cells and activated immune cells in pancreatic tissue release large amounts of oxygen free radicals, leading to increased levels of MDA and decreased levels of SOD and total GSH.[29] Reduced GSH has an important antioxidant effect. The GSSG/GSH ratio is a reliable indicator of oxidative stress, because it reflects the balance between antioxidant status and pro-oxidative reactions in the cell. The pancreas is one of the tissues with the highest concentrations of GSH in the body, and despite the relatively low activity of glutamate glutamate-cysteine ligase, this tissue shows an active cross-sulfur pathway and GSH synthesis. The lack of GSH in pancreatic tissue is a sign of the early stages of AP and can even lead to progression from mild to SAP. Studies have shown that loss of GSH may lead to premature activation of digestive enzymes in acinar cells, thereby initiating the inflammatory process.[16,30,31] To date, we only found that SLC7A11 expression was decreased in ARNT-knockout AP mice and SAP-induced AKI, and how it affects AP remains unclear.[3,4]

In genetically engineered mice, researchers found that deletion of a systemic X_c^- subunit, SLC7A1, induced tumor-selective ferroptosis and inhibited pancreatic ductal adenocarcinoma (PDAC) growth[29] [Figure 1]. However, a large number of experiments are needed to verify whether changes in GSH affect AP through the X_c^- GSH-Gpx4 pathway in ferroptosis.[16]

Iron metabolism

Iron and ferroptosis

Iron is an essential element for the human body, but excessive iron can cause various cell deaths, including ferroptosis. Theoretically, increasing the concentration of free intracellular iron by interfering with any level of iron metabolism (including its absorption, storage, utilization, and outflow) can cause ferroptosis.[21] However, although ferroptosis is iron-dependent, until recently, the exact role of iron in ferroptosis was unclear. Iron plays a variety of roles in ferroptosis. First, as a key component of a series of metabolic enzymes and protein complexes that produce energy, iron is indispensable in these oxidation-based metabolic processes and is the source of cellular ROS production. Second, iron is a cofactor of LOXs and P450s, and these two enzymes are necessary for phospholipid peroxide biosynthesis. Third, iron promotes lipid peroxidation by catalyzing the non-enzymatic Fenton reaction. When the functions of Gpx4, FSP, and/or GCH are inhibited or depleted, this chain reaction will lead to the rapid production of phospholipid peroxides and ultimately lead to ferroptosis.[11,14] Ferroptotic cells show a necrosislike morphology. The damage-associated molecular patterns (DAMPs) released from ferroptotic cells may act as extracellular inflammatory mediators and cause tissue damage.[27] In addition, iron can oxidize the mitochondrial outer membrane protein Tom20, cleave gasdermin E, and then cause pyrolysis.[28] It is unclear why only iron, but not other metals (such as zinc) that also cause ROS production through a Fenton reaction, has the ability to induce ferroptosis. One possibility is that iron overload activates specific downstream effectors, which contribute to the occurrence of ferroptosis after the production of lipid ROS.[9]

Iron homeostasis in AP

L-arginine-induced mice have higher pancreatic iron levels, suggesting that iron overload in the pancreas may play an important role in the pancreatic injury.[4] Furthermore, an animal study showed that high-iron diets or conditional knockout of Gpx4 in the pancreas promoted experimental pancreatitis in mice induced by the administration of cerulein or L-arginine (a conditionally essential amino acid). In contrast, liproxstatin- (a ferroptosis inhibitor) reversed this type of pancreatic inflammatory damage, suggesting a pathogenic role for ferroptosis in experimental pancreatitis.[17,32] In fact, the effect of iron and ferroptosis in sepsis has been described in myocardial injury, mainly due to the following reasons: (1) induction of increased GPX4; (2) reduced antioxidant; and (3) increased LIP.[33] Furthermore, growing evidence indicates that the exocrine pancreas has a bidirectional relationship with iron metabolism. During AP, excess levels of iron produce ROS leading to pancreatic β-cell failure and insulin resistance and leading to new-onset diabetes. In addition, there is evidence that pancreatic β-cells participate in iron regulation through secretion of hepcidin, and insulin has a regulatory effect on iron uptake.[34-36] At present, there is no direct evidence to show whether the increase in iron in AP induces the occurrence of ferroptosis, which is necessary for further study.

ROS in AP

ROS are generated by mitochondria as well as by enzymes involved in lipid metabolisms, such as lipoxygenases, cyclooxygenase, cytochrome P450, and NADPH oxidases.[11] ROS can be used as a signal molecule to trigger various cell death modalities, including ferroptosis. The superoxide-driven Fenton reaction catalyzed by ions is a prime source of ROS production for ferroptosis. Other sources of ROS for the induction of ferroptosis include but are not limited to the mitochondrial electron transport chain, NADPH oxidases (NOX, including NOX1, CYBB/NOX2, and NOX4), and cytochrome P450 enzyme (CYP). A continuing challenge is to distinguish whether different sources of ROS have the same efficiency in producing ferroptosis or other types of cell death. However, the precise threshold of oxidative stress that seals cell fate is largely a mystery.[21,22] To protect cells from ROS, hydroperoxides are gradually neutralized by different enzyme families, namely, lysoperoxidase dismutases, GSH peroxidases, catalases, and peroxiredoxins.[25] Mitochondrial ROS are important not only in inducing apoptosis but also in inducing ferroptosis, although the molecular switches that determine the branch between these two different types of cell death remain elusive.[9,11] In addition, once lipid peroxides are formed, they may further amplify ROS signaling and drive the mitochondrial caspase signaling pathway observed in pyroptosis, suggesting a potential connection between ferroptosis and pyroptosis. However, how these two different mechanisms work together is not fully understood. This also suggests that different RCDs in cells can interact with each other.[10] ROS also plays a key role in the pathogenesis of AP. The injured pancreatic acinar cells and activated immune cells in pancreatic tissue release large amounts of oxygen free radicals, accompanied by increased MDA and decreased SOD and total GSH levels. ROS can cause acinar cell damage, trigger the inflammatory process and lead to pancreatic edema and inflammatory cell aggregation. Moreover, antioxidant therapy has been shown to reduce acinar cell necrosis and mitigate the severity of pancreatic tissue injury in various animal models of AP.[37,38]

The p62-Kelch-Keap1-Nrf2 pathway

NRF2 is a transcription factor that regulates the expression of antioxidant proteins and protects against oxidative damage [Figure 2]. Importantly, almost all ferroptosis genes are regulated by Nrf2 transcription, including GSH regulation, NADPH regeneration, and iron regulation. In addition, Nrf2 can indirectly regulate lipids, and its abundance is related to sensitivity to ferroptosis.[13,16]

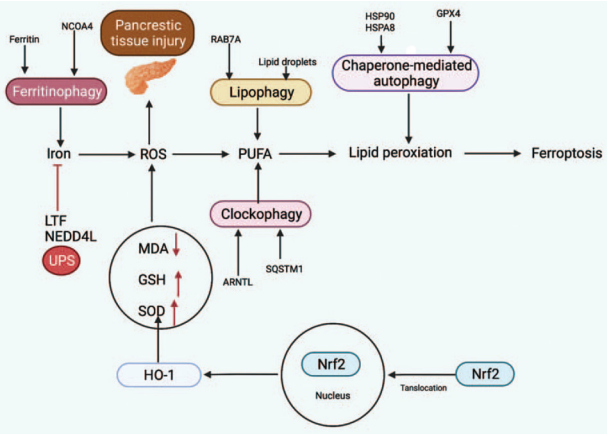

Figure 2: Role of autophagy in ferroptosis. The mechanism of non-selective macroautophagy/autophagy induced by ferroptosis activator. Certain selective types of autophagy (eg, ferritinophagy, lipophagy, clockophagy, and mitophagy) and CMA.[8,10,21,25,28,48]. CMA: Chaperone-mediated autophagy; GSH: Glutathione; HO-1: Heme oxygenase-1; MDA: Malondialdehyde; Nrf2: Nuclear factor erythroid 2-related factor; ROS: Reactive oxidative stress; SOD: Superoxide dismutase.

It is important that the up-regulation of NRF2 can increase the expression of heme oxygenase-1 (HO-1), and HO-1 is also a fully functional factor that inhibits ferroptosis.[39] Multiple studies have revealed that activation of the Keap1-Nrf2 pathway plays a significant role in the development of AP. By activating Nrf2 in the nucleus, pancreatic damage due to AP is ameliorated.[39] HO-1 is a stress-induced enzyme that plays an important role in maintaining iron homeostasis, regulating and inhibiting inflammation, and is considered to be an antioxidant and cytoprotective agent.[40] During AP, the number of macrophages increases, and HO-1 is highly expressed, so that they are recruited into the pancreas and protect the pancreas from damage. A recent study showed that increasing the expression of HO-1 in the early stage of AP can reduce pancreatic damage and TNF-α levels and increase the release of anti-inflammatory IL from macrophages, stellate cells, and helper T cell type 2 cells. The by-products of HO-1, such as bilirubin or CO, can protect AP by inhibiting the NF-κB inflammatory response and increasing the NRF2/HO-1 pathway or by impairing the recruitment of pro-inflammatory immune cells.[10,13] BML-111 is an endogenous lipid medium similar to lipoprotein A4. Studies have shown that BML-111 can up-regulate the expression of NRF2 and activate it. The HO-1/NQO-1 pathway protects the cell from cell death and tissue damage induced by oxidative stress, thereby improving pancreatitis and intestinal mucosal damage. Targeting this pathway is a potential treatment for AP-related intestinal injury.[41]

Other pathways related to AP

NADPH-FSP1-CoQ10 pathway

The NADPH-FSP1-CoQ10 pathway and GSH-Gpx4 pathway play a parallel role and can inhibit ferroptosis. FSP1 (formerly, AIFM2) can protect cells from ferroptosis by reducing CoQ10, which is a free radical scavenger.[18] CoQ10, also known as ubiquinone, represents a widely expressed family of coenzymes. Mitochondrial CoQ10 inhibits apoptosis, whereas non-mitochondrial CoQ10 prevents ferroptosis. Apoptosis-inducing factor mitochondria-associated 2 (AIFM2/FSP1) may determine the position-dependent role of CoQ10 in apoptosis and ferroptosis.[22,24] Researchers also found that administration of Q10 can attenuate pancreatic damage and related pulmonary complications by inhibiting inflammatory cytokines and inflammatory cell infiltration. Q10 may cause extracellular signal-regulated kinase and c-jun NH2-terminal kinase inactivation of AP attenuation.[42]

GCH1-BH4 pathway

The GCH1-BH4 pathway is another Gpx4-independent ferroptosis blocking pathway involving the GCH1 gene, which is the rate-limiting step in the production of the metabolite tetrahydrobiopterin (BH4).[17] BH4 is a cellular non-enzymatic redox-sensitive antioxidant. It is a precursor of neurotransmitters (eg, dopamine and serotonin) and nitric oxide (NO). Although dopamine and NO also have the ability to regulate the ferroptotic response, BH4 produced by the rate-limiting enzyme GCH1 inhibits ferroptosis through its antioxidant properties. Using BH4 and GCH1$^{-/\sim}$ mice to further explore the role of ferroptosis in neurodegenerative diseases and inflammatory diseases (such as pancreatitis induced by L-arginine (the biological precursor of NO).[22,24]

The nuclear protein 1 and transcriptional regulator 1-lipocalin 2 (NUPR1-LCN2) pathway

Some researchers have found that in *in vitro* or preclinical mouse models, inhibition of NUPR1-LCN2 pathways can enhance the anticancer activity of ferroptosis activators in PDAC cells. NUPR1 is a stress-induced transcription factor. The expression of LCN2 mediated by NUPR1 prevents ferroptosis in cells by reducing iron accumulation and subsequent oxidative damage. Lcn2$^{Pan-/-}$ mice were more sensitive to L-arginine-induced AP and hence mortality increases, pancreatic histological damage is aggravated, serum amylase (a diagnostic biomarker of AP), pancreatic myeloperoxidase (MPO, a marker of neutrophil recruitment), serum high mobility group box 1 (HMGB1), pancreatic MDA, and Ptgs2m RNA (an inducible enzyme associated with inflammation and cell death events, including ferroptosis). This Lcn2 depletion-mediated AP phenotype can be prevented by treatment with the ferroptosis inhibitor liproxstatin-1 or the iron chelator DFO. In summary, these studies suggest that LCN2 has an protective effect on AP, possibly by inhibiting the ferroptotic response[28,43] [Figure 1].

Ferroptosis and Other RCDs

Autophagy

Autophagy is defined as a catabolic process that is conserved among all eukaryotic organisms [Figure 2]. Its main functions are to degrade cytoplasmic content and recover damaged organs and proteins to maintain intracellular homeostasis when cells face stress factors such as starvation.[44] Our current knowledge on autophagy broadly differentiates it into three types: macroautophagy,

microautophagy, and chaperone-mediated autophagy (CMA).[45] Recent studies have shown that autophagy is not only involved in the inflammatory regulation of SAP and the activation of pancreatic proteinogen, but also related to the severity and prognosis of SAP.[2,46]

Macroautophagy is a cell protection mechanism that processes and recycles various cytoplasmic contents that are aged, defective, or damaged. The efficiency of protein production by acinar cells is very high, and impaired autophagy can cause trypsinogen activation and ER and mitochondrial dysfunction, making acinar cells more susceptible to other damage and death.[2,47] Various RCDs are not independent of each other. Ferroptosis is also closely related to autophagy and necroptosis.[48] Key ferroptosis repressors, such as SLC7A11 and GPX4, are degraded by the UPR.[11] Knockdown of SLC7A11 or GPX4 can attenuate autophagy and protect against ferroptosis caused by Golgi stress.[10] Furthermore, many autophagy-related genes can also activate ferroptosis; for example, inhibiting the ATG5 and ATG7 genes can reduce the accumulation of free iron and inhibit ferroptosis.[8] The relationship between ferroptosis and autophagy mainly has the following aspects.

(1) *Ferritinophagy:* The term ferritinophagy is used to describe the removal of the major iron storage protein ferritin by the autophagy machinery. NCOA4 is a cytoplasmic autophagy receptor, used to bind ferritin for subsequent degradation by ferritinophagy.[14] NCOA4 recruits ferritin into autophagosomes for lysosomal degradation to increase free iron and induce ferroptosis. Ferritin and ferroptosis are two newcomers related to iron in a variety of human diseases. They should be included in the design and evaluation of nutritional and pharmaceutical interventions for cancer, neurodegeneration, erythropoiesis, and AP treatment. This may be a promising strategy to reverse the disease.[15,28,42]

(2) *Lipophagy:* Another example of the autophagy-ferroptosis relationship is lipophagy induction, where lipid droplets (mediated by the receptor RAB7A) are degraded into free fatty acids that can be oxidized. The up-regulation of TPD52 promotes the formation of lipid droplets, thereby preventing ferroptosis caused by lipid peroxidation. On the other hand, elevated levels of RAB7A can cause activation of lipid swallowing, thereby stimulating lipid peroxidation-mediated ferroptosis.[33,48]

(3) *Mitophagy:* The selective degradation of mitochondria removes dysfunctional organelles, reduces ROS levels, prevents lipid peroxidation, and reduces the efficiency of ferroptosis. So far, >10 receptors involved in mitosis have been identified, including sequestosome 1 (SQSTM1), optineurin (OPTN), oiled-coildomain 2 (CALCOCO2), and Tax1-binding protein 1.[11,48]

(4) *Clockophagy:* That is, the autophagic degradation of the key circadian clock protein ARNTL depending on the cargo receptor SQSTM1/p62, markedly promotes ferroptosis through EGLN2/PHD1 (eglnine homolog 2/hypoxia-inducible factor prolyl hydroxylase 1)-mediated oxidative injury. Disruption of the ARNTL pathway in AP can improve the activity of ferroptosis activators *in vitro* and *in* vivo[3,10] [Figure 1].

(5) CMA: CMA involves a selective pathway that directly degrades cytoplasmic proteins in the lysosomes. CMA starts when heat shock protein family A (Hsp70) member 8 (HSPA8/HSC70) binds to proteins with a KFERQ-like motif, and then delivers the targeted proteins to lysosome-associated membrane protein type 2A (LAMP2A) on lysosomes for degradation. Heat shock protein 90 directly interacts with LAMP2A at the lysosomal membrane and preserves the stability of LAMP2A for CMA activation.[8,48]

Necroptosis

Necroptosis is mediated by receptor-interacting protein kinases (RIPs), including RIP1-RIP3, and the mixed lineage kinase-like (MLKL) pathway. MLKL is phosphorylated by RIP3, leading to its oligomerization. Inhibition of the RIP1-RIP3 pathway through gene regulation or necrostatin (an inhibitor of RIP1) can reduce the severity of acinar cell injury, so it is a potential target for AP therapy.[2,47] Studies have found that in the pathophysiological process of AKI, ferroptosis and necroptosis alternate and have a synergistic effect. In the initial stage of AKI ferroptosis is critical, whereas necroptosis takes over in the later stages, leading to enlargement of tubular cell death.[16,49] Recent studies have shown that ferroptosis plays a key role in SAP-related kidney damage. Iron accumulation and lipid peroxidation in rat kidney tissue increased during SAP. These changes are accompanied by decreased Gpx4 activity and the up-regulation of ferroptosis-related proteins and genes. By inhibiting ferroptosis, SAP-related oxidative stress, renal dysfunction, and histological damage can be improved. This means that ferroptosis may become a new target for SAP treatment.[3,4,25] Application of the specific ferroptosis inhibitor LIP-1 can not only reduce the levels of inflammatory factors and lipid peroxidation but also improve pancreatic tissue damage and renal function.[9]

Repair of cell membrane

One of the signs of ferroptosis is membrane oxidative damage, which can be repaired by at least two mechanisms. The first is to limit lipid peroxidation by activating specific enzyme systems, such as GPX4 and AIFM2. Once the first defense system fails, cells can repair damaged cell membranes through vesicle transport, exocytosis, and endocytosis.[9,10] Pore-forming proteins, such as gasdermin D (GSMD) and MLKL, mediate membrane damage in pyroptosis and necroptosis, respectively. Whether there is a similar poreforming protein-mediated membrane rupture of ferroptotic cells remains to be clarified.[11,13] There are at least three hypotheses that can answer these questions: (1) LOOH accumulation can change the integrity of the membrane and change its biophysical properties; (2) LOOH may change the location or function of membrane-associated proteins; (3) LOOH degradation into highly active products may help in the permeabilization of membranes, which may be directly toxic.[11,24,25] The endosomal sorting complexes required for transport III (ESCRT-III) machinery appear to be a common

membrane repair mechanism that counteracts various forms of regulated necrosis, including necroptosis, pyroptosis, and ferroptosis. The ESCRT complex is composed of five subcomplexes (ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III, and VPS4).[9,50] Ca^{2+} influx is a trigger for the recruitment and activation of ESCRT-III in the cell membrane during ferroptotic damage.[10] Interestingly, we also found downregulated mRNA expression of CHMP5, a core component of ESCRT-III, in L-arginine-induced $ARNTL^{Pan-/-}$ mice.[3] Current studies have shown that ESCRTI II-mediated plasma membrane repair can reduce lipid peroxidation and damage during ferroptosis (eg, HMGB1), providing a new mechanism for regulating the anticancer activity of ferroptosis activators.[11]

Ferroptosis and Immunity

The immunological consequences offerroptosis include two aspects. First, ferroptosis can lead to the death of leukocyte subsets and the corresponding loss ofimmune function. For example, lipid peroxidation-induced ferroptosis in T cells promotes viral or parasitic infections. Second, and more importantly, when ferroptosis affects nonleukocytic cells, it determines how dying cells or the resulting corpses are handled by the immune system. Different types of cell death can lead to different immune and inflammatory reactions through the release and activation of different DAMP signals. In general, ferroptosis is a form of inflammatory cell death associated with the release of DAMPs (eg, HMGB1 and DNA) or lipid oxidation products (eg, 4HNE, oxPLs, leukotriene B4, LTC4, LTD4, and prostaglandin E2) during tissue injury or tumor therapy.[11,3,14]

Conclusion and Outlook

Ferroptosis is a unique form of cell death caused by iron-dependent phospholipid peroxidation, which is regulated by a variety of cellular metabolic pathways. Many organ damage and degenerative diseases are caused by ferroptosis. Therefore, pharmacological regulation to induce and inhibit ferroptosis has great potential in the treatment of drug-resistant cancers, ischemic organ damage, and other degenerative diseases. However, there are still many questions that have not been answered: (1) What are the mechanisms by which ferroptosis is executed downstream of phospholipid peroxidation? (2) What are the physiological and pathological functions of ferroptosis in human health and disease? and (3) How do we define the crosstalk between ferroptosis, autophagy, and other types of RCD? In the next step, it will be important to understand the physiological and pathological background of ferroptosis, under which conditions and how it is induced, and whether the manipulation of ferroptosis is beneficial to the treatment and prognosis of AP.

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Conflicts of interest

None.

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