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# Myocardial Ischemia/Reperfusion Injury: Mechanism and Targeted Treatment for Ferroptosis

**ABSTRACT**

Myocardial ischemia/reperfusion injury (MIRI) is a pathophysiological process connected to the onset of numerous heart disorders. The pathogenesis of MIRI is complex, and it mainly involves calcium overload, classic oxidative stress, mitochondrial disorder, inflammation, microvascular disorder, and cell death. The clinical treatment options for MIRI are presently constrained, making it imperative to develop new treatment modalities. Recent studies have demonstrated that ferroptosis is the main cause of MIRI. Ferroptosis is a new type of regulated iron-dependent cell death whose mechanism and targeted therapy are anticipated to be novel therapeutic techniques for MIRI. Herein, the primary mechanism underlying ferroptosis (the 3 major metabolic routes involving iron, amino acids, and lipids, and in MIRI, the specific mechanism and therapeutic target of ferroptosis) are discussed to determine the potential therapeutic approach for MIRI.

**Keywords:** Myocardial ischemia/reperfusion injury, ferroptosis, pathological mechanism, targeted therapy

REVIEW

## INTRODUCTION

Acute myocardial infarction (AMI) is a disease with high morbidity, disability, and mortality worldwide. It imposes a heavy burden on the health care and economy of the society.[1] Myocardial infarction (MI) is caused by plaque accumulation in the coronary arteries inner lining. This reduces the heart's blood flow, leading to the death of cardiac cells as a result of an inadequate oxygen supply. Therefore, quick restoration of blood flow to the heart is the primary strategy for treating AMI. Reperfusion therapy following the onset of MI (thrombolysis, coronary artery bypass grafting, percutaneous coronary intervention, and bypass surgery) can effectively improve the heart's adequate blood supply. However, clinical and experimental studies have proven that restoring sufficient blood flow to the ischemic heart muscle further impairs energy metabolism, electrophysiology, tissue structure, and cardiac function of the damaged myocardium, causing myocardial death and increasing infarct size. This phenomenon is referred to as myocardial ischemia/reperfusion injury (MIRI).[2] Traditionally, it has been reported that MIRI causes calcium overload, oxidative stress, and inflammatory responses.[3] However, given that classic anti-oxidation and anti-inflammatory treatments have not achieved good curative effects, it is speculated that other mechanisms may be involved. Evidence from most studies has demonstrated that MIRI pathogenesis involves ferroptosis, and several ferroptosis indicators significantly influence the prognosis of MIRI.[4] Recently, numerous *in vitro* and *in vivo* studies have shown that ferroptosis has a therapeutic effect on various cardiovascular disorders, including MIRI.[5] Therefore, investigating the primary mechanism, function, and target of ferroptosis in MIRI may reveal new avenues for treating MIRI.

## MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

### Overview of Myocardial Ischemia/Reperfusion Injury

Reperfusion-induced myocardial injury may account for nearly half of the final myocardial injuries in AMI.[3] Cardiomyocytes are highly differentiated terminal

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cells that cannot generally divide and proliferate. These cells are permanently lost and do not regenerate once they die, which reduces the number of cardiomyocytes in an adult heart. As a consequence, a reduction in cardiomyocytes causes structural and functional changes in the heart. Therefore, inhibiting myocardial cell death is the key to protecting cardiac function and preventing heart disease.[6] For this reason, research into the mechanisms of MIRI is a hotspot topic in this field.

### Myocardial Ischemia/Reperfusion Injury Mechanism

The pathological pathway of MIRI is complex and completely unclear but mainly includes calcium overload, oxidative stress, inflammatory response, mitochondrial permeability transition pore opening, energy metabolism disorders, and activation of different cell death modes.[3] Studies have indicated that MIRI is associated with various modes of cell death, including apoptosis, necrosis, autophagy, and ferroptosis. Ferroptosis is a critical factor in these processes (Figure 1).

## MYOCARDIAL ISCHEMIA/REPERFUSION INJURY AND FERROPTOSIS

### Overview of Ferroptosis

Ferroptosis is a novel iron-dependent cell death process. Iron homeostasis is essential for cell survival and numerous biological activities. Low, high, or abnormal iron distributions throughout the body affect physiological functions. Ferroptosis is recognized as a regulated cell death (RCD) process promoted by iron overload, which was first presented by Dixon in 2012.[7] Ferroptosis is a recently discovered type of RCD mainly mediated by reactive oxygen species (ROS) groups and iron ion-mediated lipid peroxidation. The above processes are distinct from those of apoptosis, pyroptosis, necrosis, and autophagy in terms of morphology, biochemistry, genetics, and metabolism.[8] 1) Morphological features are mainly manifested as mitochondrial abnormalities (a reduction in mitochondrial volume, high mitochondrial membrane density, and fewer or absence of mitochondrial cristae), although the cell membrane is usually complete, with normal nucleus size and unconcentrated chromatin. 2) The biochemical characteristics are iron accumulation, causing lipid peroxidation. 3) The genetic characteristics are regulated by various genes, including ceruloplasmin, transferrin receptor 1 (TFR1), divalent metal-ion transporter-1 (DMT1), glutathione peroxidase 4 (GPX4), lysophosphatidylcholine acyltransferase 3 (LPCAT3), and lipoxygenase (LOX). 4) Metabolic characteristics: several metabolic pathways contribute to ferroptosis pathogenesis, including amino acid metabolism, iron metabolism, lipid metabolism, and others (Figure 2).

### HIGHLIGHTS

- Ferroptosis (characterized by iron accumulation and lipid peroxidation) is a novel mechanism of regulated cell death that plays a role in myocardial ischemia/reperfusion injury (MIRI).
- This article provides a summary from the perspectives of iron metabolism, amino acid metabolism, lipid metabolism, and antioxidation mechanisms.
- This review demonstrates that inhibiting ferroptosis may be an effective strategy for treating MIRI, and this creates new ideas for improving the clinical treatment of patients.

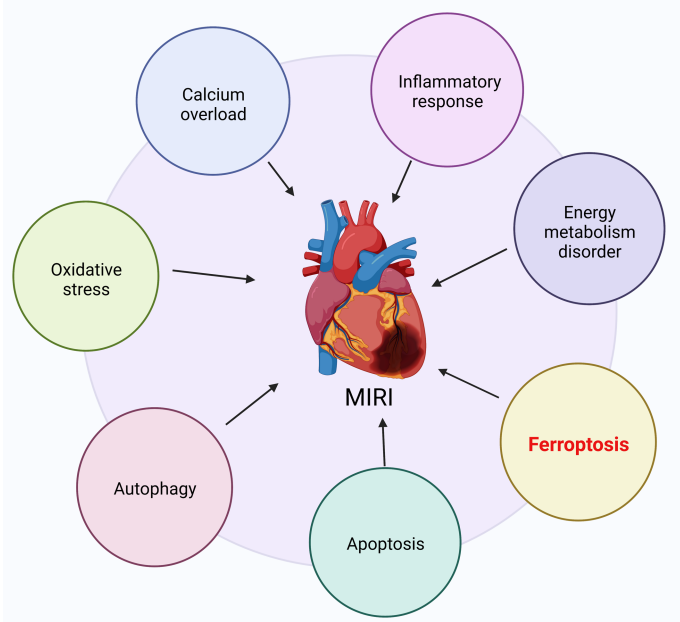


**Figure 1. Pathogenesis of MIRI. The pathological pathway of MIRI is complex, and it mainly includes calcium overload, oxidative stress, inflammatory response, mitochondrial permeability transition pore opening, energy metabolism disorders, and activation of different cell death modes (including apoptosis, necrosis, autophagy, and ferroptosis). Ferroptosis is a critical factor in these processes. MIRI, myocardial ischemia/reperfusion injury.**

### Metabolic Pathways of Ferroptosis

The ferroptosis mechanism is very complex and comprises a sequence of signaling and metabolic pathways. Iron, amino acids, and lipid metabolism are vital for ferroptosis pathogenesis. The principal metabolic pathways in these 3 types of ferroptosis are discussed in the following sections (Figure 3).

### Iron Metabolism—Iron-Mediated Ferroptosis

Ferroptosis is intricately linked to the presence of iron. Iron, a vital trace element in the human body, is crucial for maintaining normal physiological functions. Thus, any disruption to iron homeostasis, whether due to iron overload or deficiency, can lead to various disorders, eventually causing ferroptosis.[9]

### Iron Import

Generally, iron can infiltrate cells in 2 different forms: transferrin-bound iron and non-transferrin-bound iron. Ferric iron ($Fe^{3+}$) is absorbed by transferrin-bound iron. First, the ferroxidase activity of copper-containing glycoprotein ceruloplasmin converts ferrous iron ($Fe^{2+}$) to $Fe^{3+}$.[10] Second, TFR1 allows extracellular $Fe^{3+}$ to enter the cell after binding to transferrin,

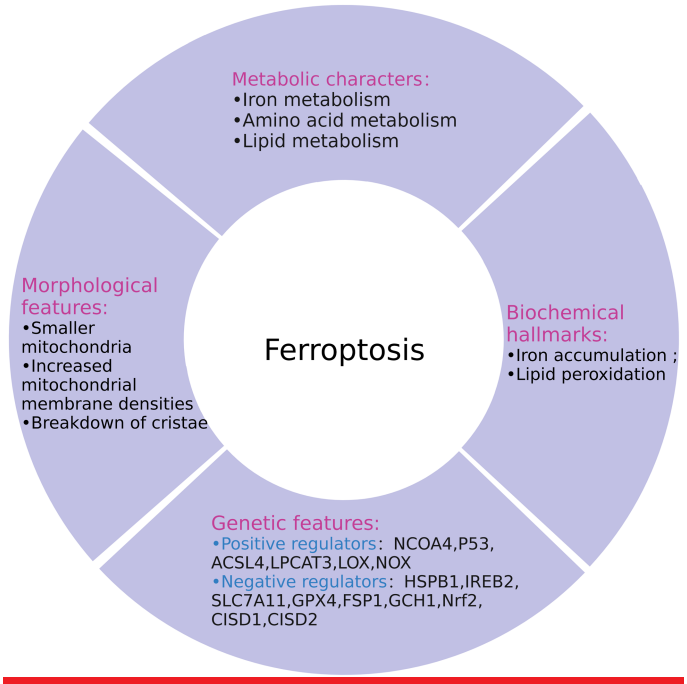


**Figure 2. Characteristics of ferroptosis (morphological, biochemical, genetic, and metabolic characteristics).**

which is the primary iron uptake pathway.[8] In addition, non-transferrin-bound iron is primarily absorbed into cells in $Fe^{2+}$ form, increasing the level of cardiac labile iron.[9] L-type voltage-dependent calcium channel,[6] T-type voltage-dependent calcium channel, metal transporter SLC39A14,[11] DMT1, and zinc transporter are imported into cardiomyocytes. Heat shock protein B1 inhibits TFR1, decreases iron ion concentrations, and inhibits ferroptosis.[12] Overexpression of ubiquitin-specific protease 7, which mediates ferroptosis, activated the p53/TFR1 pathway in rats with MIRI (Figure 3) (Table 1).[13]

### *Iron Transfer and Storage*

Endosomes store $Fe^{3+}$ within cells, and iron reductase 6 transmembrane epithelial antigens of prostate 3 transforms $Fe^{3+}$ to $Fe^{2+}$.[8] DMT1 then facilitates $Fe^{2+}$ passage from endosomes to the cytoplasmic labile iron pool (LIP), with the remaining iron stored in ferritin (FT).[14] Labile iron pool is a pool of iron complexes that are chelatable and redox-active. When LIP concentration attains a steady-state limit, it initiates peroxidative reactions, including Fenton and Haber–Weiss reactions. Peroxidative reactions cause severe damage, generate ROS, and activate lipoxin synthases, causing lipid peroxidation of polyunsaturated fatty acids ((PUFAs); making up biological membranes), which are the current underlying mechanisms for ferroptosis initiation. Ferritin, the most common iron-storing protein, is a globular hetero-poly protein with 24 FT heavy chain (FTH) and FT light chain (FTL) components. Ferritin heavy chain converts $Fe^{2+}$ to $Fe^{3+}$ and stores it in the spherical hollow structure of FT, lowering levels of free iron, while FTL is responsible for the storage of direct iron. Iron response element binding protein 2 promotes FTL and FTH-1 expression, decreases intracellular iron levels, and suppresses erastin-induced ferroptosis.[7] The nuclear receptor coactivator 4 transports FT into autophagosomes, where it undergoes lysosomal degradation to release free iron, which increases cellular susceptibility to ferroptosis (ferritinophagy).[14,15] Generally, iron autophagy maintains iron homeostasis under normal physiological conditions. Overactivation of iron autophagy causes excessive iron deposition in cells, inducing ferroptosis. Lactoferrin mediates iron transport, promoting ferroptosis through iron accumulation (Figure 3) (Table 1).[16]

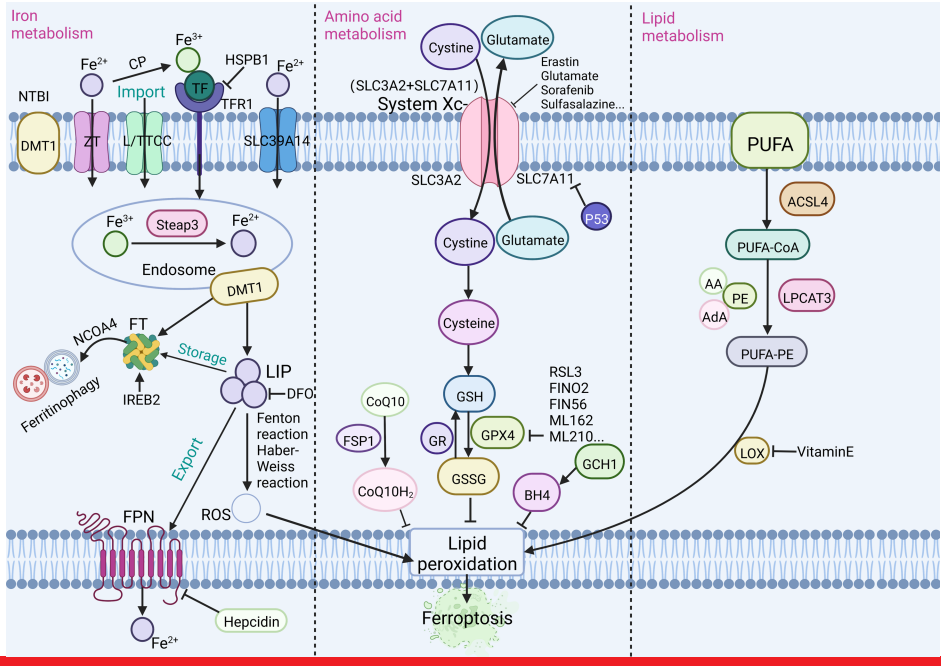


**Figure 3. Schematic diagram of the ferroptosis mechanism.**

**Table 1. The Ferroptosis Regulatory Genes**

| | | Regulatory Genes | Function |
|---|---|---|---|
| Iron metabolism | Positive regulator | *CP* | Creates $Fe^{3+}$ from $Fe^{2+}$ and facilitates iron binding with transferrin |
| | | *TF* | Siderophore |
| | | *TFR1* | Intake of extracellular $Fe^{3+}$ into the cell |
| | | *SLC39A14* | Transports iron that is not bound by transferrin |
| | | *DMT1* | Takes extracellular $Fe^{2+}$ into the cell; mediates the liberation of $Fe^{2+}$ from the endosome into LIP |
| | | *Steap3* | Reduces $Fe^{3+}$ in the endosome to $Fe^{2+}$ |
| | | *NCOA4* | Trafficking of ferritin into autophagosomes for lysosomal breakdown and free iron release |
| | | *USP7* | Activates the p53/TFR1 pathway |
| | Negative regulator | *HSPB1* | Inhibits TFR-1, thereby reducing cellular iron uptake |
| | | *FTH1* | Stores intracellular iron |
| | | *IREB2* | Enhances expression of FTL and FTH-1 |
| | | *Prominin2* | Mediates ferritin export |
| | | *mTOR* | Downregulates transferrin receptor 1 and increases FPN expression |
| | | *Nrf2* | Regulates iron metabolism, antioxidant response |
| | | *CISD1* and *CISD2* | Regulates mitochondrial iron |
| Amino acid metabolism | Positive regulator | *GLS2* | Converts glutamine to glutamate |
| | | *P53* | Down-regulates SLC7A11 and inhibits cystine uptake by System $Xc^-$ |
| | | *YTHDF2* | Inhibits SLC7A11 |
| | Negative regulator | *SLC7A11* | Facilitates the absorption of cystine and the release of glutamate |
| | | *SLC3A2* | Maintains the stability of SLC7A11 |
| | | *GPX4* | Inhibits lipid peroxidation |
| | | *USP22* | via the SIRT1/p53/SLC7A11 axis |
| | | *FSP1* | Increases content of antioxidants BH4 and CoQ10 |
| | | *GCH1* | Reduction of coenzyme Q10 to ubiquinone |
| Lipid metabolism | Positive regulator | *ACSL4* | Converts PUFA to PUFA-CoA |
| | | *LPCAT3* | PUFA-CoA subsequently combines with PE via LPCAT3 to form PUFA-PE |
| | | *LOXs* | Promotes lipid peroxidation |
| | Negative regulator | *YAP* | Transcriptionally activates NEDD4L to cause the ubiquitination and destruction of ACSL4 |

### Iron Export

Two proteins, ferroportin-1 (FPN1) and prominin 2 (PROM2), are primary mediators of iron export.

The membrane protein FPN1 exports iron from the cell, generating $Fe^{3+}$ from $Fe^{2+}$. Hepcidin, a 25-amino acid protein predominantly secreted from hepatocytes, modulates FPN. Hepcidin stimulates FPN internalization and breakdown, decreasing iron efflux from cardiomyocytes and promoting ferroptosis (Figure 3) (Table 1).[17]

Prominin 2 mediates FT export. Brown et al[18] found that PROM2 (a 5-transmembrane glycoprotein) mediates the FT-containing multivesicular bodies as well as exosomes (EXOs) formation. The multivesicular structures and EXOs expel iron from the cells, effectively reducing ferroptosis (Figure 3) (Table 1).

## AMINO ACID METABOLISM: INHIBITION OF LIPID PEROXIDATION

### System Xc−-glutathione-GPX4 Axis—Classic Ferroptosis Suppression Route

System $Xc^-$/glutathione (GSH)/GPX4 axis is the primary ferroptosis suppression mechanism. This axis contains 2 ferroptosis key regulators, including GSH and GPX4. System $Xc^-$ is a glutamate/cystine antiporter and a heterodimer containing 2 subunits (solute carrier family 7 member 11 (SLC7A11) and solute carrier family 3 member 2 (SLC3A2)), mainly through SLC7A11 (the main active site of erastin). System

$Xc^-$ releases glutamate from the cell to the outside at a ratio of 1 : 1 across the cell membrane and also absorbs cystine throughout the cell from outside and is decreased to cysteine in the cell. Glutam, cysteine, and glycine are used to produce GSH. GPX4, a selenoprotein and a vital antioxidant enzyme, inhibits lipoxygenase and cyclooxygenase activity during ferroptosis. GPX4, which relies on GSH, transforms reduced GSH to oxidized glutathione (GSSG) and lowers toxic lipid peroxide (LPO) to non-toxic lipid alcohol. GPX4 removes excess peroxide and hydroxyl radicals secreted during cellular respiration and metabolism. Consequently, GPX4 can precisely and effectively eliminate phospholipid hydroperoxide, suppressing ferroptosis. Suppression of the system Xc− mechanism reduces intracellular GSH levels, decreasing GPX4 activity and ferroptosis. A high extracellular glutamate concentration suppresses the antiporter system Xc− and promotes ferroptosis, which indicates the toxicity of high glutamate accumulation in the nervous system (Figure 3) (Table 1).[8]

Glutaminase (GLS1 and GLS2) transforms glutamine to glutamate during ferroptosis, regulating extracellular glutamate concentration. GLS2 is included in the initiation of ferroptosis. Suppression of glutaminase activity can effectively inhibit ferroptosis and alleviate MIRI (Table 1).[19] Studies have demonstrated that GPX4 of mitochondrial-specific overexpression attenuates cardiac malfunction following the onset of ischemia/reperfusion. Suppression of ferroptosis results in suppression of glutaminolysis (a GSH synthesis pathway component), which potentially alleviates cardiac damage caused by I/R.

The tumor suppressor P53 downregulates SLC7A11 and limits cystine absorption by system $Xc^-$, impacting GPX4 action and diminishing the antioxidant ability of cells. These accumulate lipid ROS, causing ferroptosis (Figure 3) (Table 1).[20]

YT521-B homology domain family member 2 has the ability to inhibit SLC7A11, which interferes with glutamate and cystine transport and lowers GPX4 production. However, iron excess may result from this. These 2 factors synergize to induce ferroptosis and exacerbate MIRI.[21]

Ubiquitin-specific protease 22 prevents ferroptosis-stimulated cardiac cells death in MIRI by sirtuin-1/p53/SLC7A11 axis (Table 1).[22]

Moreover, nicotinamide adenine dinucleotide phosphate (NADPH) further regulates ferroptosis by maintaining GSH in a reduced state (Figure 3).[23]

Several compounds and drugs can induce ferroptosis. Currently, ferroptosis promoters are divided into 2 groups according to whether they directly inactivate GPX4. Class 1 ferroptosis promoters suppress the system Xc−, whereas class 2 ferroptosis promoters directly suppress GPX4.

Erastin, a class 1 ferroptosis promoter, is one of the principal ferroptosis inducers. Erastin blocks system Xc− and the absorption of cystine into cells. This decreases GSH production and indirectly turns off GPX4, causing LPOs to accumulate to promote ferroptosis. Moreover, Erastin engages and suppresses the voltage-dependent anion channels VDAC2 and VDAC3, causing mitochondrial malfunction and contributing to massive oxide discharge, ultimately triggering iron-mediated cell death.[24] More inhibitors of system Xc− include glutamate, sorafenib, sulfasalazine, imidazolone, Istin, interferon-γ, and diarylisoxazole.[6]

Class 2 ferroptosis promoters, including RSL3, directly block GPX4, promote lipid peroxidation, and induce ferroptosis. RSL3 does not affect GPX4 upstream, such as GSH depletion and cysteine uptake. Subsequently, more direct GPX4 inhibitors were identified, such as FINO2, ferroptosis inducing 56, ML162 (DPI7), and ML210 (DPI10). Ferroptosis inducing 56 causes ferroptosis by depleting coenzyme Q10 (CoQ10) and promoting GPX4 degradation.[6]

## Ferroptosis Suppressor Protein 1-CoQ10 Axis—An Emerging Pathway to Suppress Ferroptosis

The FSP1-CoQ10 pathway is a GPX4-independent pathway that inhibits ferroptosis and aligns with GPX4 and GSH in inhibiting LPO proliferation and ferroptosis (Figure 3).[25]

Suppression of ferroptosis via FSP1 is promoted by CoQ10. Myristoylation attracts FSP1 to the plasma membrane, where it acts as an oxidoreductase that decreases CoQ10 with NAD(P)H and forms a lipophilic free radical trapping antioxidant, inhibiting production of LPO (Table 1).

## Guanosine triphosphate cyclohydrolase 1- Tetrahydrobiopterin/Dihydrobiopterin (BH4/BH2)-Phospholipid Axis—An Emerging Pathway to Suppress Ferroptosis

GCH1-BH4/BH2 is another GPX4-independent pathway that suppresses ferroptosis. Using a CRISPR-mediated genome-wide activation screen, Kraft et al[26] revealed that GCH1 and its metabolic products BH4/BH2 are essential for gradual resistance of ferroptosis (Figure 3).

GCH1 is the rate-limiting enzyme in BH4 production. Upregulation or silencing of GCH1 causes corresponding resistance or sensitivity of cells to ferroptosis through modulating the antioxidant BH4 endogenous production, which is mechanistically capable of selectively preventing tailed phospholipid autooxidation and CoQ10 production. In turn, CoQ10 confers BH4 with an emerging lipophilic free radical trapping antioxidant that requires dihydrofolate reductase (Table 1).

## Lipid Metabolism: Lipid Peroxide Lead to Ferroptosis

The key factors causing ferroptosis include LPO accumulation, specifically the transformation of PUFAs into LPO through oxidation. Arachidonoyl (AA) containing PUFA and adrenoyl phospholipids are major components of biofilms, and the primary substrates for ferroptosis lipid peroxidation are PUFA-containing phospholipids (Figure 3).[27]

So far, 3 enzymes involved in phospholipid metabolism have been identified to participate in ferroptosis. These enzymes contain acyl-CoA synthetase long-chain family member 4 (ACSL4), LPCAT3, and LOX, which are involved in the biosynthesis, remodeling, and oxidation of phosphatidylethanolamine (PE), respectively (Figure 3).[27]

PUFA is acylated as a result of the catalysis of ACSL4 to generate PUFA acyl-coenzyme A (PUFA-CoA).[27] PUFA-CoA subsequently combines with PE via LPCAT3 to form PUFA-PE. Polyunsaturated fatty acid-phosphatidylethanolamine is oxidized by LOX to lipid hydroperoxides, which initiates ferroptosis. The production of intracellular LPO substrates decreases with the downregulation of ACSL4, LPCTA3, and LOX, preventing ferroptosis. ACSL4 in the above process is considered more effective than LPCTA3. Lipid peroxidation and ferroptosis are fundamentally influenced by the lipoxygenase family (LOXs), iron-containing enzymes which catalyze the PUFA dioxygenation), particularly 12/15-LOX.[4,27] Lipoxygenase stimulates ferroptosis by modulating the oxidation of AA and adrenal-esterified phosphatidylethanola mine throughout the endoplasmic reticulum. The small scaffold protein phosphatidylethanolamine-binding protein 1, a suppressor of the protein kinase cascade, binds to 15-LOX to promote ferroptosis (Figure 3) (Table 1).[28]

A member of the E3 ubiquitin ligase family, neural precursor cell expressed developmentally downregulated 4-like (NEDD4L), plays a critical role in the emergence of numerous disorders by aiding in the ubiquitination of its target proteins. Studies have demonstrated that NEDD4L regulates the pathogenesis of various cardiovascular disorders. The Hippo pathway's essential component is the yes-associated protein (YAP). To reduce ferroptosis during MIRI, YAP transcriptionally activated NEDD4L to cause the ubiquitination and destruction of ACSL4.[29]

### Regulators Related to the Ferroptosis Metabolic Pathway

The processes included in ferroptosis involve the metabolism of iron, amino acids, and lipids. Related positive and negative regulators participate in ferroptosis through various pathways. Genes that play essential roles are listed in Table 1.

### Nuclear Factor Erythroid 2-Related Factor 2

Nuclear Factor Erythroid 2-Related Factor 2 triggers the activation of numerous essential target genes included in modulating ferroptosis through regulating amino acid metabolism, iron metabolism, lipid metabolism, and mitochondrial function (Table 1).[6]

Nuclear Factor Erythroid 2-Related Factor 2 is a protein that has a significant function in iron homeostasis, regulating several genes included in iron metabolism [FTH, FTL, transferrin, FPN, and heme oxygenase-1 (HO-1)].[17,30]

Moreover, Nrf2 regulates amino acid metabolism in ferroptosis. Nuclear Factor Erythroid 2-Related Factor 2, a transcription factor, serves as a pivotal regulator of cellular antioxidant responses. Under stimulation of oxidative stress, Nrf2 translocates to the nucleus and forms a heterodimer with Maf protein, after which it starts the target gene transcription that has antioxidant response elements. Subsequently, superoxide dismutase, glutathione peroxidase, and catalase production are initiated, lowering oxidative stress, the inflammatory response, and ferroptosis. Studies have indicated that Nrf2 suppresses ferroptosis by modulating SLC7A11 and HO-1 expression.[31]

### Heme Oxygenase-1

The HO-1 effect on ferroptosis remains to be validated. Heme Oxygenase-1 is a crucial enzyme in heme catabolism that catalyzes heme degradation into $Fe^{2+}$, carbon monoxide (CO), and biliverdin. The overactivity of HO-1 increases LIP, subsequently triggering ferroptosis.[32] However, moderate overexpression of HO-1 protects the cells because of its antioxidant activity. HO-1 and metabolites of heme metabolism (biliverdin and CO) have antioxidant capabilities. Heme Oxygenase-1 activation can reduce oxidative stress and exert anti-apoptotic and anti-inflammatory characteristics, enhancing myocardial resistance to I/R injury. Preconditioning of donor rats using inhaled CO prevents I/R injury after heart transplantation.[17]

### CDGSH Iron-Sulfur Domains 1 and CDGSH Iron-Sulfur Domains 2 (CISD2)

Ferroptosis is negatively regulated by the mitochondrial iron regulators CISD1 and CISD2. The inhibition of CISD1 or CISD2 contributes to ferroptosis (Table 1).[33,34]

## FERROPTOSIS IN MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

The cardiotoxic effects of iron, coupled with its correlation to reperfused MI in individuals with iron overload in the peri-infarct region, underscore the importance of investigating ferroptosis in MIRI. Numerous studies have revealed that excess iron is related to the pathophysiology of MIRI. For instance, myocardial I/R activates hypoxia-inducible factor 1 signaling and upregulates TFR1 expression to promote iron absorption and increase iron content.[14] In a 30-minute occlusion experiment for I/R damage in a mice model, the left anterior descending coronary artery (LAD) was not perfused by blood. This resulted in FT accumulation in the myocardial scar region and increased cardiac cytosolic iron levels in I/R due to the mobilization of high iron levels into the coronary blood stream after extended ischemia.[35] Similarly, mice with MIRI had elevated mitochondrial iron.[14] In an experiment of the ischemic area of the heart, scarce alterations in iron, GPX4, ACSL4, or malondialdehyde (MDA) were detected. However, in a reperfusion mouse model, iron, ACSL4, and MDA contents were increased, and GPX4 expression was downregulated.[36] The above findings suggest that ferroptosis occurs during myocardial reperfusion, not during ischemia. This implies it could be a novel approach for therapeutic intervention in MIRI.

### Ferroptosis Treatment in Myocardial Ischemia/Reperfusion Injury

Ferroptosis have a vital function in the pathogenesis of MIRI, consequently, ferroptosis inhibition is a possible MIRI therapeutic target.[4]

Ferroptosis inhibitors mainly consist of iron chelators [deferoxamine (DFO), deferiprone (DFP), and dexrazoxane (DXZ) etc.] and lipid peroxidation inhibitors [e.g., ferrostatin-1, liproxstatin-1, vitamin E, N-acetylcysteine (NAC), CoQ10, and XJB-5-131]. The effects and mechanisms underlying some ferroptosis inhibitors in MIRI are summarized below.

Iron chelators decrease iron levels, preventing ferroptosis in vivo and in vitro. In most cases, DFO (which binds to free iron, treats iron overload, and stops ferroptosis caused by lipid peroxidation) is a harmless iron chelator. Studies have revealed that DFO prevents I/R injury in isolated hearts. Another study demonstrated that DFO administration prior to reperfusion via primary percutaneous coronary intervention significantly decreased oxidative stress but did not diminish infarct size.[37] Paraskevaidis et al[38] demonstrated that intravenous infusion of DFO during coronary artery bypass grafting prevented reperfusion damage of the myocardium and decreased lipid peroxidation.

Deferoxamine (DFP) targets hemorrhagic iron in I/R injury and provides cardioprotective benefits in AMI via attenuating intramyocardial bleeding and cardiac hypertrophy.[39] In addition, DFP monotherapy is more cardioprotective than treatment with DFO only and subcutaneous DFO therapy.[6]

Dexrazoxane prevented doxorubicin-induced ferroptosis in mice, reducing fatal cardiac injury (infarct size) and myocardial dysfunction after I/R.[35]

Although iron chelating agents have achieved certain results in treating myocardial I/R injury, there are also some unsatisfactory aspects. The positive therapeutic effect of iron chelators on MIRI is not evident in some experimental animals, which may be attributed to the species specificity of iron chelators.[14]

P22077 (a suppressor of ubiquitin-specific protease 7) has been demonstrated to prevent MIRI via suppressing ferroptosis by p53 overexpression and TFR1 downregulation.[40]

The dark red pigment echinochrome A (found in sea urchin shells and spines) is lipophilic and membrane permeable. A water-soluble version of echinochrome A, known as histochrome, exhibits potent iron-chelating and antioxidant properties. Histochrome therapy chelated intracellular iron, significantly caused overexpression of GPX4 and free GSH, and downregulated cyclooxygenase 2 expression to confer cardioprotection against MIRI.[41]

Polydopamine nanoparticles (PDA NPs) inhibited $Fe^{2+}$ accumulation and restored mitochondrial function in H9c2 cells. Polydopamine nanoparticles efficiently decreased $Fe^{2+}$ deposition and lipid peroxidation in a mouse model of MIRI. Furthermore, PDA NPs treatment reduced MIRI throughout the mouse model, manifested diminished infarct size, and promoted cardiac function. The current investigation demonstrated the therapeutic effect of PDA NPs on MIRI by stopping ferroptosis.[42]

In a previous study, ponatinib and DFO suppressed ferroptosis and necroptosis in myocardial I/R, and their incorporation significantly lowered myocardial infarct size.[43] In another recent study, DXZ and ponatinib were shown to decrease ferroptosis during MIRI. Combining the above 2 drugs significantly reduced MI scar size. These results suggest that a combined therapy targeting distinct forms of cell death modalities is an efficient treatment approach for MIRI.

Lip-1 prevents I/R injury in mouse cardiac muscles. Lip-1 administration after ischemia can lower MI size. Lip-1 suppresses ferroptosis via upregulating GPX4, reducing ROS, lowering VDAC1 expression, and supporting mitochondrial structure and function following myocardial I/R.[44]

In a mouse model of MIRI, the glutaminolysis inhibitor compound 968 inhibited ferroptosis, reduced MI size, and improved cardiac function.[19]

Cysteine has been acetylated to become NAC. N-acetylcysteine is a GSH donor and exhibits antioxidant effects. GSH is a substrate used by enzymes to reduce other compounds as part of the endogenous antioxidant system. In addition, GSH functions directly as an antioxidant. As a result, the above medication may be utilized to augment antioxidant therapy when GSH levels are decreased in a hyperoxidative condition.[45]

Pretreatment with either Fer-1 or DXZ lessens MI severity by preventing myocardial enzyme elevations resulting from I/R.[35]

MIRI significantly affects heart transplantation. I/R injury in heart transplantation can result in considerable sterile inflammation, causing primary graft malfunction in patients and significantly increasing the mortality rate. Investigation of the correlation between ferroptosis and cardiac transplantation-associated I/R injury demonstrated that ferroptosis attracts neutrophils to the damaged myocardium when damage-associated molecular patterns are released. Fer-1 decreases cardiomyocyte mortality through a mechanism involving damage-associated molecular patterns and prevents neutrophil attraction to damaged cardiomyocytes after heart transplantation.[46] Therefore, focusing on ferroptosis suppression offers a therapeutic avenue for treating individuals at high risk of developing MIRI after coronary blood flow fixation after a heart transplant. Suppressing ferroptosis in the donor heart before surgery may also reduce MIRI and improve the prognosis.

Etomidate inhibits ferroptosis in MIRI model by upregulating Nrf2 and HO-1 expression.[47]

Dexmedetomidine (Dex) significantly attenuates MI, improves cardiac function, and reduces hypoxia/reoxygenation-induced $Fe^{2+}$ accumulation and lipid peroxidation in cardiomyocytes. Dexmedetomidine substantially suppressed ferroptosis via upregulation of Nrf2, SLC7A11, and GPX4 expression.[48]

Ferroptosis is inhibited by XJB-5-131 (a synthetic antioxidant derived from nitrogen oxides), which directs mitochondria and creates ROS and electron scavenging capabilities.[49]

Activation of endoplasmic reticulum stress (ERS) exacerbates cardiomyocyte ferroptosis. A study in MIRI model (generated by ligating the LAD in diabetic rats) demonstrated that infusion of Fer-1 into the tail vein ameliorated ERS-induced cardiomyocyte ferroptosis. Furthermore, salubrinal (an inhibitor of ERS) alleviated cardiomyocyte ferroptosis.[50]

## CONCLUSION AND OUTLOOK

Myocardial ischemia/reperfusion injury is one of the crucial reasons for morbidity, disability and mortality globally. Understanding the pathological process of cardiomyocyte injury is critical for establishing cardioprotective strategies. Ferroptosis is associated with MIRI and modulates MIRI by amino acid metabolism, iron metabolism, lipid metabolism, ERS, and autophagy-dependent ferroptosis mechanisms.

Many challenges underlying the mechanism and treatment of MIRI are yet to be investigated. The majority of current research on suppressing ferroptosis to alleviate MIRI shed light on the animal model and cell culture, with limited estimation for its clinical effectiveness. Therefore, a transition towards clinical research is necessary. In addition to ferroptosis, MIRI involves numerous different mechanisms in the cell death pathway. Detailed investigations of ferroptosis and different modes of RCDs have revealed a comprehensive link between ferroptosis and other RCDs. However, the predominant kind of cell death in MIRI remains to be validated. Consequently, more investigations are necessary to determine the function of distinct cell death pathways in developing MIRI. As MIRI worsens because of numerous different types of cell death, combining therapies that target distinct cell death types may be the best way to prevent MIRI progression. Studies have demonstrated that the incidence of iron death varies at various stages of MIRI, but the exact stage at which iron death occurs has not been clarified. Cell-secreted EXOs have become a hotspot area due to their immunogenic defects. Therefore, the interaction between EXOs and ferroptosis is a future research hotspot.

In conclusion, ferroptosis showed the potential to an efficient therapeutic target for MIRI. However, further investigation is required to fully uncover its molecular mechanism and potential role in this condition.

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## REFERENCES

1. Tsao CW, Aday AW, Almarzooq ZI, et al. American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke Statistics-2023 update: A report from the American Heart Association. *Circulation*. 2023;147(8);39(918):e93-e621. [CrossRef]. Erratum in: *Circulation*. Erratum. 2023;147(8):e622 Erratum in: *Circulation*. 2023;148(4):e4. (https://doi.org/10.1161/CIR.0000000000001167)
2. Ibáñez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. *J Am Coll Cardiol*. 2015;65(14):1454-1471. [CrossRef]
3. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest*. 2013;123(1):92-100. [CrossRef]
4. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol*. 2021;22(4):266-282. [CrossRef]
5. Wu X, Li Y, Zhang S, Zhou X. Ferroptosis as a novel therapeutic target for cardiovascular disease. *Theranostics*. 2021;11(7):3052-3059. [CrossRef]
6. Li N, Jiang W, Wang W, Xiong R, Wu X, Geng Q. Ferroptosis and its emerging roles in cardiovascular diseases. *Pharmacol Res*. 2021;166:105466. [CrossRef]
7. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*. 2012;149(5):1060-1072. [CrossRef]
8. Fang X, Ardehali H, Min J, Wang F. The molecular and metabolic landscape of iron and ferroptosis in cardiovascular disease. *Nat Rev Cardiol*. 2023;20(1):7-23. [CrossRef]
9. Anderson GJ, Frazer DM. Current understanding of iron homeostasis. *Am J Clin Nutr*. 2017;106(suppl 6):1559S-1566S. [CrossRef]
10. Shang Y, Luo M, Yao F, Wang S, Yuan Z, Yang Y. Ceruloplasmin suppresses ferroptosis by regulating iron homeostasis in hepatocellular carcinoma cells. *Cell Signal*. 2020;72:109633. [CrossRef]
11. Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ. Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. *Proc Natl Acad Sci U S A*. 2006;103(37):13612-13617. [CrossRef]
12. Sun X, Ou Z, Xie M, et al. HSPB1 as a novel regulator of ferroptotic cancer cell death. *Oncogene*. 2015;34(45):5617-5625. [CrossRef]
13. Tang LJ, Zhou YJ, Xiong XM, et al. Ubiquitin-specific protease 7 promotes ferroptosis via activation of the p53/TfR1 pathway in the rat hearts after ischemia/reperfusion. *Free Radic Biol Med*. 2021;162:339-352. [CrossRef]
14. Li S, Zhang X. Iron in cardiovascular disease: challenges and potentials. *Front Cardiovasc Med*. 2021;8:707138. [CrossRef]
15. Gao M, Monian P, Pan Q, Zhang W, Xiang J, Jiang X. Ferroptosis is an autophagic cell death process. *Cell Res*. 2016;26(9):1021-1032. [CrossRef]
16. Wang Y, Liu Y, Liu J, Kang R, Tang D. NEDD4L-mediated LTF protein degradation limits ferroptosis. *Biochem Biophys Res Commun*. 2020;531(4):581-587. [CrossRef]
17. Ravingerová T, Kindernay L, Barteková M, et al. The molecular mechanisms of iron metabolism and its role in cardiac dysfunction and cardioprotection. *Int J Mol Sci*. 2020;21(21):7889. [CrossRef]
18. Brown CW, Amante JJ, Chhoy P, et al. Prominin2 drives ferroptosis resistance by stimulating iron export. *Dev Cell*. 2019;51(5):575-586.e4. [CrossRef]
19. Gao M, Monian P, Quadri N, Ramasamy R, Jiang X. Glutaminolysis and transferrin regulate ferroptosis. *Mol Cell*. 2015;59(2):298-308. [CrossRef]
20. Jiang L, Kon N, Li T, et al. Ferroptosis as a p53-mediated activity during tumour suppression. *Nature*. 2015;520(7545):57-62. [CrossRef]

21. Pang P, Si W, Wu H, et al. YTHDF2 promotes cardiac ferroptosis via degradation of SLC7A11 in cardiac ischemia-reperfusion injury. *Antioxid Redox Signal*. 2023. [CrossRef]
22. Ma S, Sun L, Wu W, Wu J, Sun Z, Ren J. USP22 protects against myocardial ischemia-reperfusion injury via the SIRT1-p53/SLC7A11-dependent inhibition of ferroptosis-induced cardiomyocyte death. *Front Physiol*. 2020;11:551318. [CrossRef]
23. Shimada K, Hayano M, Pagano NC, Stockwell BR. Cell-line selectivity improves the predictive power of pharmacogenomic analyses and helps identify NADPH as biomarker for ferroptosis sensitivity. *Cell Chem Biol*. 2016;23(2):225-235. [CrossRef]
24. Yagoda N, von Rechenberg M, Zaganjor E, et al. RAS-RAF-MEK-dependent oxidative cell death involving voltage-dependent anion channels. *Nature*. 2007;447(7146):864-868. [CrossRef]
25. Bersuker K, Hendricks JM, Li Z, et al. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature*. 2019;575(7784):688-692. [CrossRef]
26. Kraft VAN, Bezjian CT, Pfeiffer S, et al. GTP cyclohydrolase 1/tetrahydrobiopterin counteract ferroptosis through lipid remodeling. *ACS Cent Sci*. 2020;6(1):41-53. [CrossRef]
27. Li D, Li Y. The interaction between ferroptosis and lipid metabolism in cancer. *Signal Transduct Target Ther*. 2020;5(1):108. [CrossRef]
28. Wenzel SE, Tyurina YY, Zhao J, et al. PEBP1 wardens ferroptosis by enabling lipoxygenase generation of lipid death signals. *Cell*. 2017;171(3):628-641.e26. [CrossRef]
29. Qiu M, Yan W, Liu M. YAP facilitates NEDD4L-mediated ubiquitination and degradation of ACSL4 to alleviate ferroptosis in myocardial ischemia-reperfusion injury. *Can J Cardiol*. 2023;39(11):1712-1727. [CrossRef]:(S0828-282X(23)01573-8)
30. Kerins MJ, Ooi A. The roles of NRF2 in modulating cellular iron homeostasis. *Antioxid Redox Signal*. 2018;29(17):1756-1773. [CrossRef]
31. Fan Z, Wirth AK, Chen D, et al. Nrf2-Keap1 pathway promotes cell proliferation and diminishes ferroptosis. *Oncogenesis*. 2017;6(8):e371. [CrossRef]
32. Kwon MY, Park E, Lee SJ, Chung SW. Heme oxygenase-1 accelerates erastin-induced ferroptotic cell death. *Oncotarget*. 2015;6(27):24393-24403. [CrossRef]
33. Yuan H, Li X, Zhang X, Kang R, Tang D. CISD1 inhibits ferroptosis by protection against mitochondrial lipid peroxidation. *Biochem Biophys Res Commun*. 2016;478(2):838-844. [CrossRef]
34. Kim EH, Shin D, Lee J, Jung AR, Roh JL. CISD2 inhibition overcomes resistance to sulfasalazine-induced ferroptotic cell death in head and neck cancer. *Cancer Lett*. 2018;432:180-190. [CrossRef]
35. Fang X, Wang H, Han D, et al. Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci U S A*. 2019;116(7):2672-2680. [CrossRef]
36. Tang LJ, Luo XJ, Tu H, et al. Ferroptosis occurs in phase of reperfusion but not ischemia in rat heart following ischemia or ischemia/reperfusion. *Naunyn Schmiedebergs Arch Pharmacol*. 2021;394(2):401-410. [CrossRef]
37. Chan W, Taylor AJ, Ellims AH, et al. Effect of iron chelation on myocardial infarct size and oxidative stress in ST-elevation-myocardial infarction. *Circ Cardiovasc Interv*. 2012;5(2):270-278. [CrossRef]
38. Paraskevaidis IA, Iliodromitis EK, Vlahakos D, et al. Deferoxamine infusion during coronary artery bypass grafting ameliorates lipid peroxidation and protects the myocardium against reperfusion injury: immediate and long-term significance. *Eur Heart J*. 2005;26(3):263-270. [CrossRef]
39. Behrouzi B, Weyers JJ, Qi X, et al. Action of iron chelator on intramyocardial hemorrhage and cardiac remodeling following acute myocardial infarction. *Basic Res Cardiol*. 2020;115(3):24. [CrossRef]
40. Hao S, Liang B, Huang Q, et al. Metabolic networks in ferroptosis. *Oncol Lett*. 2018;15(4):5405-5411. [CrossRef]
41. Hwang JW, Park JH, Park BW, et al. Histochrome attenuates myocardial ischemia-reperfusion injury by inhibiting ferroptosis-induced cardiomyocyte death. *Antioxidants (Basel)*. 2021;10(10):1624. [CrossRef]
42. Zhang Y, Ren X, Wang Y, et al. Targeting ferroptosis by polydopamine nanoparticles protects heart against ischemia/reperfusion injury. *ACS Appl Mater Interfaces*. 2021;13(45):53671-53682. [CrossRef]
43. Tu H, Zhou YJ, Tang LJ, et al. Combination of ponatinib with deferoxamine synergistically mitigates ischemic heart injury via simultaneous prevention of necroptosis and ferroptosis. *Eur J Pharmacol*. 2021;898:173999. [CrossRef]
44. Feng Y, Madungwe NB, Imam Aliagan AD, Tombo N, Bopassa JC. Liproxstatin-1 protects the mouse myocardium against ischemia/reperfusion injury by decreasing VDAC1 levels and restoring GPX4 levels. *Biochem Biophys Res Commun*. 2019;520(3):606-611. [CrossRef]
45. Aldini G, Altomare A, Baron G, et al. N-acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. *Free Radic Res*. 2018;52(7):751-762. [CrossRef]
46. Li W, Feng G, Gauthier JM, et al. Ferroptotic cell death and TLR4/Trif signaling initiate neutrophil recruitment after heart transplantation. *J Clin Invest*. 2019;129(6):2293-2304. [CrossRef]
47. Lv Z, Wang F, Zhang X, Zhang J, Liu R. Etomidate attenuates the ferroptosis in myocardial ischemia/reperfusion rat model via Nrf2/HO-1 pathway. *Shock*. 2021;56(3):440-449. [CrossRef]
48. Wang Z, Yao M, Jiang L, et al. Dexmedetomidine attenuates myocardial ischemia/reperfusion-induced ferroptosis via AMPK/GSK-3β/Nrf2 axis. *Biomed Pharmacother*. 2022;154:113572. [CrossRef]
49. Krainz T, Gaschler MM, Lim C, Sacher JR, Stockwell BR, Wipf P. A mitochondrial-targeted nitroxide is a potent inhibitor of ferroptosis. *ACS Cent Sci*. 2016;2(9):653-659. [CrossRef]
50. Li W, Li W, Leng Y, Xiong Y, Xia Z. Ferroptosis is involved in diabetes myocardial ischemia/reperfusion injury through endoplasmic reticulum stress. *DNA Cell Biol*. 2020;39(2):210-225. [CrossRef]