

Role of Ferroptosis in Fibrotic Diseases

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Abstract: Ferroptosis is a unique and pervasive form of regulated cell death driven by iron-dependent phospholipid peroxidation. It results from disturbed cellular metabolism and imbalanced redox homeostasis and is regulated by various cellular metabolic pathways. Recent preclinical studies have revealed that ferroptosis may be an attractive therapeutic target in fibrotic diseases, such as liver fibrosis, pulmonary fibrosis, kidney fibrosis, and myocardial fibrosis. This review summarizes the latest knowledge on the regulatory mechanism of ferroptosis and its roles in fibrotic diseases. These updates may provide a novel perspective for the treatment of fibrotic diseases as well as future research.

Keywords: ferroptosis, liver fibrosis, kidney fibrosis, myocardial fibrosis, pulmonary fibrosis

Introduction

Fibrosis is defined by excessive accumulation of an extracellular matrix (ECM) such as collagen and fibronectin.¹ Pathophysiologically, fibrosis is a dysregulated pathologic extension of the normal wound healing response to repetitive or chronic tissue damage, which is characterized by injury, inflammation, myofibroblast activation and migration, and matrix deposition and remodeling.^{2,3} Common organs involved in fibrosis include the liver (cirrhosis, non-alcoholic steatohepatitis), kidney (chronic kidney disease, renal interstitial fibrosis), heart (heart failure, cardiomyopathy), lung (idiopathic pulmonary fibrosis, cystic fibrosis), and skin (scleroderma),^{4,5} representing a high incidence rate and significant disease burden. Progressive fibrosis leads to disruption of functional tissue architecture and ultimately organ failure.^{5,6} Currently available antifibrotic treatments have limited clinical benefits for prevention or regression of disease progression,⁴ therefore, it is of great significance to explore the effective strategies for fibrosis based on well-established underlying mechanisms. In the past decade, there has been a growing appreciation for the importance of ferroptosis in explaining the pathophysiology of fibrosis and its regression. However, ferroptosis in different cell types may exert distinct influence on fibrosis. The extent to which ferroptosis affects fibrotic diseases is unclear, although several studies have found significant correlations between ferroptosis-associated genes and pathways and fibrotic diseases. Herein, we present the key molecular mechanisms of ferroptosis, describe the crosstalk between ferroptosis and fibrosis-associated signaling pathways, and provide insights into the potential novel therapeutic target of ferroptosis in the context of fibrotic disorders.

Mechanisms of Ferroptosis

Ferroptosis has been a rapidly evolving research area since it was coined in 2012⁷ (see [Figure 1](#)). The Nomenclature Committee on Cell Death (NCCD) defines ferroptosis as “a form of regulated cell death (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”.⁸ Subsequent investigations identified a wide range of inducers and inhibitors of ferroptosis (see [Table 1](#)). This unique RCD, driven by iron-dependent lipid peroxidation (LPO), is caused by redox imbalance between oxidative damage and antioxidant defense.⁹ As discussed below, various cellular metabolic pathways have been reported continually in the regulation of ferroptosis at multiple levels, including

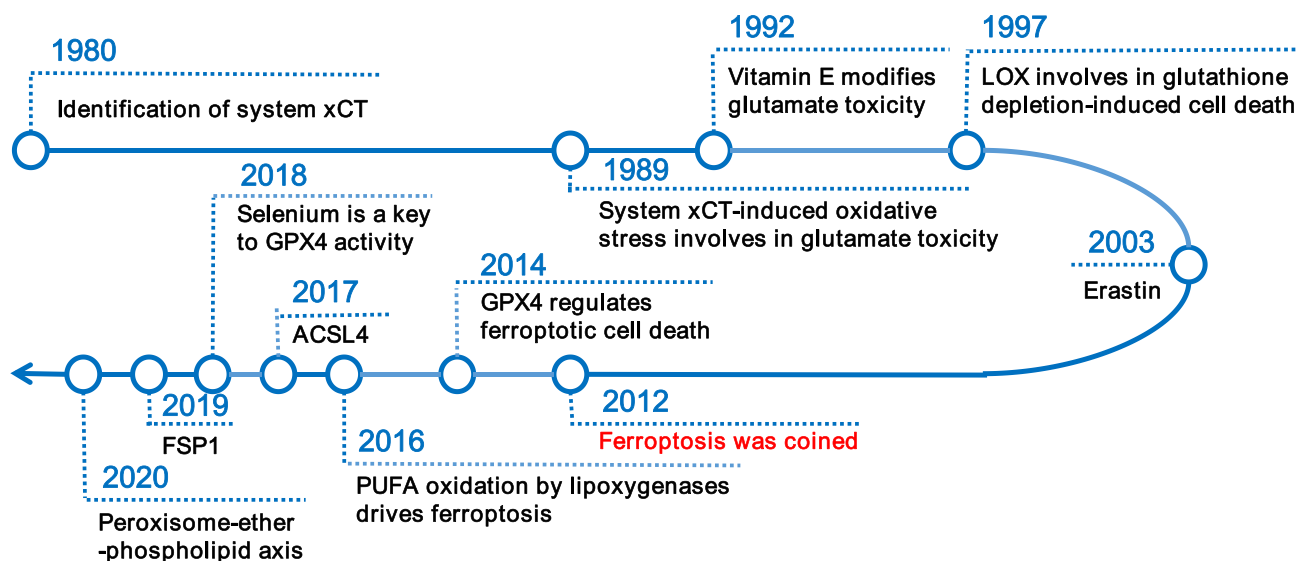


Figure 1 Overview of research history of ferroptosis.

Abbreviations: xCT, cystine/glutamate antiporter; LOX, lipoxygenase; GPX4, glutathione peroxidase 4; ACSL4, Acyl-CoA synthetase long-chain family member 4; PUFA, polyunsaturated fatty acid; FSP1, ferroptosis suppressor protein 1.

iron handling, lipid metabolism, mitochondrial activity, redox homeostasis, membrane repair, and intracellular degradation.

Iron-Mediated Oxidative Stress

Excessive iron deposition has been reported in fibrotic diseases both in preclinical experimental studies and in clinical samples.^{10–13} The trace element iron plays central roles in many biological processes of ferroptosis. On the one hand, Fenton reaction relies on iron to amplify phospholipid hydroperoxides (PLOOHs), and PLOOHs further react with iron to yield free radicals and propagate a peroxidation chain reaction;⁷ on the other hand, iron-dependent enzymes such as lipoxygenases (LOXs), cytochrome P450 oxidoreductase (POR), and NADPH oxidase and many other reactive oxygen species (ROS)-generating processes require iron for catalysis.¹⁴ The intracellular labile iron pool (LIP) is carefully orchestrated primarily by iron-regulatory proteins in import, storage, export, and utilization.^{15,16} Insoluble ferric (Fe^{3+}) from dietary intakes binds to transferrin (Trf) and lactotransferrin, two iron-binding transport proteins that positively regulate iron uptake.^{17,18} Cellular iron is mainly imported by the plasma membrane protein transferrin receptor (TFRC)-mediated endocytosis and stored within ferritin.¹⁹ Specifically, in the acidic environment of endosomes, ferrous reductase STEAP3 metalloredoxase reduces Fe^{3+} to ferrous (Fe^{2+}), the free form of which constitutes LIP.¹⁶ Then solute carrier family 11 member 2 (SLC11A2/DMT1) mediates the release of Fe^{2+} from endosome to cytoplasm. Ferritin heavy chain (FHC) and ferritin light chain constitute the iron-storage protein ferritin, the degradation of which by lysosomes increases LIP. The export of cellular iron is mediated by ferroportin 1 coupled with ceruloplasmin or by ferritin-containing multivesicular bodies and exosomes.¹⁹ In extracellular space, Fe^{2+} is oxidized to Fe^{3+} by ferroxidases.¹⁶ Conceivably, ferroptosis can be promoted by increasing cellular iron import or releasing ferritin-stored iron; on the contrary, processes that enhance cellular iron export have been shown to suppress ferroptosis (see Figure 2).

LPO-Mediated Cytotoxicity

It is now established that uncontrolled LPO is the hallmark of ferroptosis.^{20,21} The oxidative destruction of lipids requires transition metal iron, ROS and phospholipids containing polyunsaturated fatty acid chains (PUFA-PLs).²⁰ Briefly, initiation of LPO can occur by both non-enzymatic and enzymatic way in PUFA-PLs. Enzymatic LPO is catalyzed by LOXs and POR;^{22,23} Non-enzymatic LPO is driven by Fenton reaction, a chemical reaction between iron and hydrogen peroxide generating PLOOHs.²⁴ Polyunsaturated fatty acids (PUFAs) such as arachidonic acid and adrenic acid are the

Table 1 Inducers and Inhibitors of Ferroptosis

Effect	Mode of Action	Molecular/Compound
Inducers	Cystine/glutamate antiporter inhibition	Erastin, Piperazine erastin, Imidazole ketone erastin, Sorafenib, Sulfasalazine, Glutamate
Inducers	Glutathione depletion	Buthionine sulfoximine, Cystine/cysteine deprivation, High extracellular glutamate, Cyst(e)inase, Cisplatin, Multidrug resistance protein 1, Cytosolic GSH-degrading enzyme CHAC1, Artesunate
Inducers	Glutathione peroxidase 4 inhibition	RAS-selective lethality protein 3 (RSL3), FIN56, FINO ₂ , ML162, ML210, Altretamine, JKE-1674, DPI compounds
Inducers	Disruption of enzymes of the transsulfuration pathway	Glycine N-methyltransferase, S-adenosyl homocysteine hydrolase, Cystathionine β-synthase, Cystathionine γ-lyase
Inducers	Block CoQ10 synthesis	Statins (cerivastatin, simvastatin), iFSP1
Inducers	Promote ROS production	Ferroptocide (Inhibition of thioredoxin), L-glutamate, α-ketoglutarate
Inducers	Promote lipid peroxidation	Dietary ω-6 polyunsaturated fatty acid
Inducers	Increase labile iron pool	Hemin, Hemoglobin, Ferric ammonium citrate, Dihydroartemisinin, Siramesine and lapatinib
Inhibitors	Iron depletion	Deferoxamine, Ciclopirox, Deferiprone, Heat shock protein family B (small) member 1, CISD1, CISD2, PCBPI, Prominin2
Inhibitors	Lipid peroxidation inhibition	Ferrostatis, Liproxstatis, CoQ10, Idebenone, Butyl-hydroxytoluene, Tetrahydrobiopterin, Dihydrobiopterin, Nitric oxide, XJB-5-131, JP4-039, Rosiglitazone (ACSL4 inhibitor), Triacsin C, Deuterated polyunsaturated fatty acid, Butylated hydroxytoluene, Butylated hydroxyanisole, Monounsaturated fatty acids, Palmitoleic acid or oleate, Squalene, Cu-ATSM
Inhibitors	Restraining lipoxygenases	Vitamin E, alpha-tocopherol, Trolox, Tocotrienols, PD-146176, AA-861, CDC, Baicalein, Zileuton, MK886, LOXBlock-1
Inhibitors	Inhibit ROS production	Dipeptidyl-peptidase-4 inhibitors (Vildagliptin, Alogliptin, Linagliptin), DPI, GKT137831, GKT136901, Apocynin, GPNA, Compound 968, AOA
Inhibitors	Upregulate/stabilize SLC7A11 expression	ETS-1, Activating transcription factor 4, Nuclear factor E2-related factor 2, CD44, the deubiquitylase OTUB1
Inhibitors	Glutaminolysis	Glutamine deprivation, Glutaminolysis inhibitor

Abbreviations: GSH, Glutathione; CoQ10, coenzyme Q10, ubiquinol; ROS, reactive oxygen species; ACSL4, Acyl-CoA synthetase long-chain family member 4; SLC7A11, solute carrier family-7 member-11; OTUB1, OTU deubiquitinase, ubiquitin aldehyde binding 1.

main precursors of PLOOHs.²⁵ The adjacent double bond of the bisallylic methylene group in PUFAs makes it more susceptible to be oxidized than other fatty acids (saturated fatty acids [no double bond], monounsaturated fatty acids [1 double bond]) by abating the hydrogen bonding energy.²⁰ Acyl-CoA synthetase long-chain family member 4 (ACSL4) ligates long-chain PUFAs with coenzyme A.^{26,27} Then lysophosphatidylcholine acyltransferase 3 (LPCAT3) re-esterified them into phospholipids and membranes.²⁶ Therefore, ACSL4 and LPCAT3 are two critical membrane-remodeling enzymes involved in the LPO of ferroptosis (see Figure 2). In addition, a recent study of lipidomic profiling reported that polyunsaturated ether phospholipids synthesized by peroxisomes also acts as substrates for LPO.²⁸ Apart from lipid synthesis, lipid storage and degradation also affect ferroptosis sensitivity. Formation of endoplasmic reticulum-derived lipid droplets isolates PUFA from membrane phospholipids and limits ferroptosis, while lipophagy increases LPO and subsequent ferroptosis.²⁹⁻³¹

Antioxidant System-Mediated Detoxification Defenses

Ferroptosis occurs when the prerequisites for ferroptosis exceed the buffering capability of ferroptosis defense systems.⁹ As discussed below, the cyst(e)ine/glutathione (GSH)/GPX4 axis, the ferroptosis suppressor protein 1 (FSP1)/coenzyme Q10 (CoQ10, coenzyme Q10, ubiquinol) axis, the dihydroorotate dehydrogenase (DHODH)/CoQ10 axis, and the GTP cyclohydrolase 1 (GCH1)/tetrahydrobiopterin (BH₄)/dihydrofolate reductase (DHFR) axis are four known antioxidant systems that mediate detoxification of lipid peroxides and protect against ferroptosis (see Figure 2). While the cyst(e)ine/GSH/GPX4 axis locates both in cytoplasm and in mitochondria, the FSP1/CoQ10 axis mainly cooperates with cytosolic

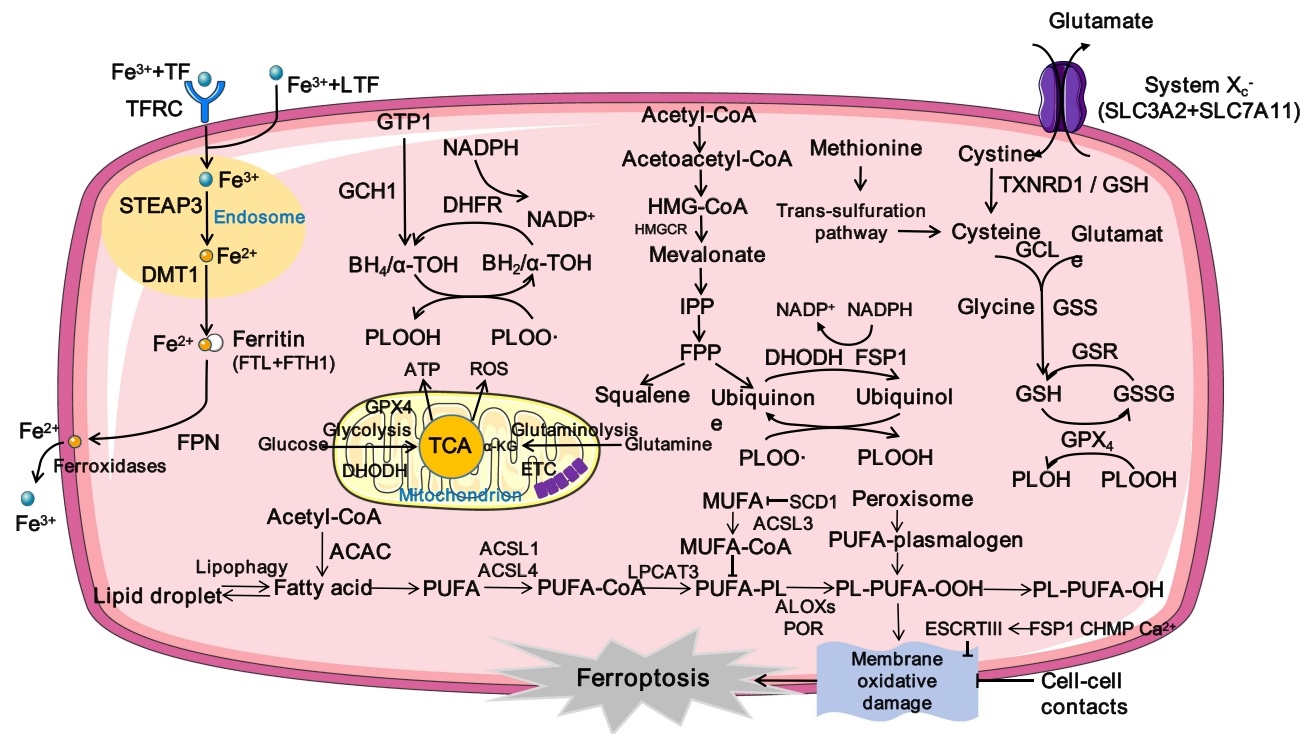


Figure 2 Mechanism underlying ferroptosis.

Abbreviations: Fe³⁺, ferric; TF, transferrin; TFRC, transferrin receptor; LTF, lactotransferrin; STEAP3, six-transmembrane epithelial antigen of the prostate 3; Fe²⁺, ferrous; DMT1, divalent metal transporter 1; FTL, ferritin light chain; FTH1, ferritin heavy chain 1; FPN, Ferroportin; GCH1, GTP cyclohydrolase 1; DHFR, dihydrofolate reductase; PLOOH, dihydroorotate dehydrogenase; PLOO, peroxy radical; PLOH, phospholipid alcohol; HMG-CoA, hydroxy methylglutaryl coenzyme A reductase inhibitor; HMGR, HMG-CoA Reductase; IPP, isopentenyl pyrophosphate; FPP, farnesyl pyrophosphate; FSP1, ferroptosis suppressor protein 1; DHODH, dihydroorotate dehydrogenase; SLC7A11, solute carrier family-7 member-11; GCL, glutamate-cysteine ligase; GSS, Glutathione Synthetase; GSR, glutathione-disulfide reductase; GSH, glutathione; GSSG, oxidized glutathione; GPX4, glutathione peroxidase 4; ATP, Adenosine triphosphate; ROS, reactive oxygen species; ETC, electron transport chain; α -KG, α -ketoglutarate; ACAC, Acetyl CoA carboxylase; PUFA, polyunsaturated fatty acid; PUFA-PLs, phospholipids containing polyunsaturated fatty acid chains; MUFA, monounsaturated fatty acid; ACSL1, Acyl-CoA synthetase long-chain family member 1; ACSL3, Acyl-CoA synthetase long-chain family member 3; ACSL4, Acyl-CoA synthetase long-chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; POR, cytochrome P450 oxidoreductase; ALOXs, lipoxygenases.

GPX4 on the plasma membrane, and the DHODH/CoQ10 axis collaborates with mitochondrial GPX4 in the mitochondria. So far the subcellular localization of the GCH1/BH4/DHFR axis is not fully-illustrated.³²

The cyst(e)ine/GSH/GPX4 axis is the first discovered and most extensively studied pathway in suppressing ferroptosis.³³ GPX4 is a selenoprotein that catalyzes the reduction and detoxification of lipid ROS production in mammalian cells.³⁴ As the major PLOOH-neutralizing enzyme, GPX4 reduces the PLOOHs to corresponding alcohols (PLOHs), with two electrons most commonly from GSH.³⁴ As the most abundant antioxidant in mammalian cells, GSH plays a critical role in the generation of iron-sulfur clusters and catalysis of GPX4 and glutathione-S-transferases.¹⁴ After GPX4-mediated reduction of PLOOH, the oxidized GSH (GSSG) will be recycled by GSH-disulfide reductase with electrons provided by NADPH/H⁺.¹⁴ Cysteine is the rate-limiting substrate for the biosynthesis of reduced GSH.³⁵ Cysteine can be (1) imported from environment: by a neutral amino acid transporter/in its oxidized form (cystine) by the system xc- cystine/glutamate antiporter (a transmembrane protein complex containing catalytic subunit solute carrier family 7 member 11 [SLC7A11] and regulatory subunit solute carrier family 3 member 2 [SLC3A2]) and further deoxidized via GSH or thioredoxin reductase 1;³⁶ or (2) synthesized using methionine and glucose in the trans-sulfuration pathway.³⁷

The FSP1/CoQ10 axis was identified in 2019 by two independent studies using an overexpression screen in one study and a synthetic lethal CRISPR-Cas9 knockout screen in the other.^{38,39} FSP1 (previously known as apoptosis-inducing factor mitochondrial 2), which is initially called p53-responsive gene 3,⁴⁰ is a target of transcription factors NRF2,⁴¹ CRBP,⁴² and PPAR α .⁴³ Ferroptosis induced by pharmacological inhibition or genetic deletion of GPX4 can be completely suppressed by FSP1, however, the anti-ferroptotic function of FSP1 disappears with the mutation of the myristoylation site.

Mechanistically, ferroptosis inhibition by FSP1 is mediated via CoQ10 in a NADPH-dependent manner. On the one hand, FSP1 functions as oxidoreductase to reduce CoQ10 to ubiquinol, which traps lipid peroxy radicals that mediate lipid autoxidation; on the other hand, FSP1 regenerates the oxidized α -tocopherol radical (vitamin E), which is an effective natural chain-breaking antioxidant.^{38,39} Similarly, DHODH is a mitochondria-localized enzyme that can also reduce CoQ10 to ubiquinol, thus detoxifying mitochondrial LPO and conferring defenses to ferroptosis in parallel to mitochondrial GPX4.⁴⁴

BH4 and dihydrobiopterin are metabolic products of GCH1.⁴⁵ Recently it has been reported that BH4 protects phospholipids containing two PUFA tails against oxidative degradation via functioning as an endogenous radical-trapping antioxidant or participating in ubiquinone synthesis.⁴⁶ In addition, endosomal sorting complexes required for transport-III-Dependent Membrane Repair systems confer resistance to oxidative damage in ferroptosis.⁴⁷ These defense systems operate synergistically or complementarily in negatively regulating ferroptosis.

Role of Ferroptosis in Fibrotic Diseases

Studies on ferroptosis or fibrosis that converge on cellular metabolism have revealed an intimate relationship between ferroptosis and fibrosis and their shared metabolic pathway. Increased glycolysis, upregulation of glutaminolysis, and enhanced fatty acid oxidation are critical drivers of metabolomic reprogramming of activated fibroblasts,² while under certain conditions tricarboxylic acid cycle fueled by glutaminolysis may play a major role in ferroptosis induction.⁴⁸ Besides, the perturbation of cellular iron and redox homeostasis involves in transforming growth factor- β (TGF- β)-induced epithelial-mesenchymal transition (EMT) during fibrosis. Dramatic decline in the FHC as well as rise in intracellular free iron during TGF- β -induced EMT contribute to iron overload, which boosts ROS production.⁴⁹ Conversely, ROS also takes part in TGF- β -induced EMT and modulates TGF- β 's fibrogenic effects through different pathways.^{50,51} Downregulated GSH in TGF- β -induced EMT of fibrosis was observed in vivo, in vitro, and in human beings.^{52–55} Additionally, upregulated LPO indicated by an increased level of malondialdehyde was reported in experimental animal models of pulmonary fibrosis.⁵⁶ The fibrosis-related pathways are illustrated in Figure 3 and summarized in Table 2. The overlaps between drugs targeting ferroptosis in fibrotic diseases are depicted in Figure 4 and summarized in Table 3.

Ferroptosis and Hepatic Fibrosis

Hepatic fibrosis refers to the replacement of damaged normal tissue by fibrous scar which is mainly composed of excessive deposition of ECM.⁵⁷ There are two main types of chronic liver injuries that lead to liver inflammation and subsequent fibrosis: hepatotoxic injury (caused by chronic injury of hepatocytes) and cholestatic injury (caused by obstruction to bile flow). The conventional experimental animal model includes pericentral liver fibrosis (acute hepatocellular injury induced by hepatocellular carbon tetrachloride) and periportal liver fibrosis (cholestasis induced by bile duct ligation). Among the multiple progenitor cells in the liver, hepatic stellate cells (HSCs) are the primary source of myofibroblast, regardless of etiology.⁵⁸ Besides, portal fibroblast, bone marrow-derived fibrocytes, mesothelial cells, and Gli+ mesenchymal stem cell-like cells can also transform into myofibroblasts.⁵ Cirrhosis is the ultimate result of hepatic fibrogenesis, which leads to hepatocarcinoma and liver failure.⁵⁷

Intriguingly, ferroptosis has been reported to have contradictory effects in the processes of progression and regression of liver fibrosis. It has long been speculated that iron deposition may be a predisposing factor for liver fibrosis and regression. Liver biopsy samples obtained from patients with liver cirrhosis showed increased iron levels and ferroptosis markers. Ferroptosis of myofibroblast induced by inhibiting cystine/glutamate antiporter (xCT)/SLC7A11 aggravates chronic liver injury, which is strongly correlated with liver fibrosis.⁵⁹ High dietary iron predisposes mice to liver fibrosis, which can be reversed by ferroptosis inhibitor ferrostatin-1 (Fer-1), suggesting that ferroptosis underlies fibrosis.^{10,60} Actually, ferroptosis-induced liver fibrosis is initiated by the excessive free non-heme iron in liver tissue. Using a hepatocyte-specific Trf knockout mice model, Yu et al¹⁰ found that loss of hepatic Trf, a serum-abundant metal-binding protein which plays a critical role in iron homeostasis, contributes to ferroptosis-induced liver fibrosis. Specifically, in the context of Trf insufficiency, Slc39a14 transports large amounts of non-Trf-bound iron into hepatocytes, thereby promotes ferroptosis-induced liver fibrosis under high dietary iron and carbon tetrachloride (CCl₄) injections.¹⁰ Iron overload promote ferroptosis in hepatocytes via heme oxygenase-1 (HO-1) pathway, which results in liver injury and fibrosis. Fibroblast growth factor 21 (FGF21) is an endocrine

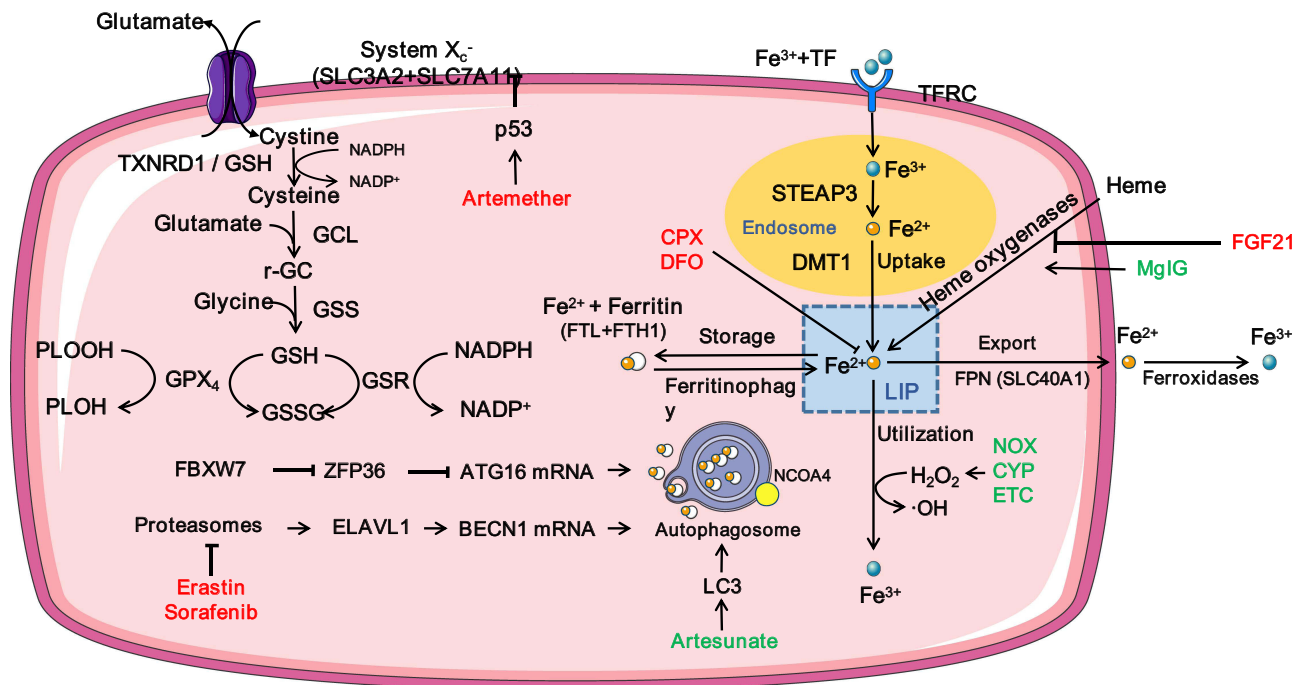


Figure 3 Fibrotic-related pathways in ferroptosis.

Abbreviations: SXC, System Xc-; SLC7A11, solute carrier family-7 member-11; GCL, glutamate-cysteine ligase; GSS, Glutathione Synthetase; GSR, glutathione-disulfide reductase; GSH, glutathione; GSSG, oxidized glutathione; GPX4, glutathione peroxidase 4; PLOH, phospholipid alcohol; PLOOH, dihydroorotate dehydrogenase; Fe³⁺, ferric; TF, transferrin; TFR, transferrin receptor; LTF, lactotransferrin; STEAP3, six-transmembrane epithelial antigen of the prostate 3; Fe²⁺, ferrous; DMT1, divalent metal transporter 1; FTL, ferritin light chain; FTH1, ferritin heavy chain 1; FPN, Ferroportin; LIP, labile iron pool; FGF21, Fibroblast growth factor 21; HO-1, heme oxygenase-1; MgIG, Magnesium isoglycyrrhizinate; CPX, Ciclopirox; DFO, deferoxamine; NOX, NADPH oxidase; CYP, cytochrome P450; ETC, electron transport chain; LC3, microtubule-associated protein light chain 3; FBXW7, F-box and WD repeat domain containing 7; ZFP36, ZFP36 ring finger protein; ATG16, autophagy related 16; ELAVL1, ELAV like RNA binding protein 1; BECN1, Beclin 1; NCOA4, nuclear receptor coactivator 4.

member of the FGF family that plays an important role in energy balance and metabolism of glucose and lipid. Wu et al⁶¹ found that FGF21 promotes HO-1 ubiquitination and degradation as well as activates nuclear factor erythroid 2-related factor 2 (Nrf2), both of which provide a mechanistic explanation for the inhibitory effects of FGF21 on ferroptosis and liver fibrosis. Nevertheless, several other studies reported that ferroptosis has an inhibitory effect on liver fibrosis, possibly by inactivating HSCs and inducing HSCs death, two main mechanisms of removal of hepatic myofibroblasts during the regression of liver fibrosis.⁵⁸ Magnesium isoglycyrrhizinate, a natural product derived from natural glycyrrhizic acid, markedly attenuated CCl₄-induced hepatic fibrosis by HO-1 mediated HSCs ferroptosis.⁶² Artesunate, a water-soluble hemisuccinate derivative of artemisinin, remarkably promoted ferroptosis of activated HSCs in a ferritinophagy-mediated way, the anti-fibrosis effect of which can be completely abolished by deferoxamine (DFO).⁶³ Consistently, Artemether (ART), another derivative of artemisinin which is the primary choice for the therapy of malaria, ameliorates liver fibrosis and HSCs activation by P53-dependent induction of ferroptosis.⁶⁴ Another study reported that iron regulatory protein 2-iron-ROS axis is also indispensable for the anti-fibrotic effect of ART via inducing ferroptosis in HSC.⁶⁵ Moreover, dihydroartemisinin, another artemisinin derivative, was also found to alleviate hepatic fibrosis through inducing ferroptosis in HSCs, which is closely related to autophagy activation.⁶⁶ Recently, it is reported that wild bitter melon extract as a potential antifibrotic agent of liver fibrosis attenuates lipopolysaccharide-induced HSCs activation via the regulation of ER stress and ferroptosis.⁶⁷

Activation and transdifferentiation of quiescent HSCs into matrix-producing myofibroblasts represented a milestone in liver fibrosis.⁵⁷ Targeting to scavenge HSCs by activating ferroptosis is considered to be a potential strategy to treat liver fibrosis. Several molecules such as ELAV like RNA binding protein 1 (ELAVL1), ZFP36 ring finger protein (ZFP36), bromodomain-containing protein 7 (BRD7), and tripartite motif-containing protein 26 (TRIM26) have been reported to play crucial roles in regulating ferroptosis in HSCs. ELAVL1, a ubiquitous RNA-binding protein which regulates mRNA cargos containing AU-rich elements (AREs) in the 3'-untranslated region (3'-UTR), regulates ferroptosis in liver fibrosis in a posttranscriptional manner. Erastin or sorafenib treatment upregulates the expression

Table 2 Molecules Involved in Ferroptosis and Fibrosis

Molecules	Function for Ferroptosis	Effect for Disease	Mechanisms	Test Cells	Disease Model	Reference
Liver fibrosis						
HO-1	Induce	Alleviate	Promote the accumulation of iron and lipid peroxides	HSC	Rat model of CCl ₄ -LF	[62]
ELAVL1	Induce	Alleviate	Promote autophagic ferritin degradation	HSC	Murine model of BDL-LF	[68]
p53	Induce	Alleviate	p53-dependent induction of ferroptosis	HSC	Murine model of CCl ₄ -LF	[64]
ZFP36	Induce	Alleviate	ZFP36 downregulation, ferritinophagy activation, and ferroptosis induction	HSC	Murine model of BDL-LF	[69]
BRD7	Induce	Alleviate	Promote p53 mitochondrial translocation	HSC	Murine model of BDL-LF	[70]
TRIM26	Induce	Alleviate	Facilitate SLC7A11 ubiquitination	HSC	Murine model of CCl ₄ -LF	[72]
m6A modification	Induce	Alleviate	Stabilizing BECN1 mRNA	HSC	Murine model of CCl ₄ -LF	[73]
NCOA4	Induce	Alleviate	Autophagy activation and upregulation of NCOA4	HSC	Rat model of CCl ₄ -LF	[66]
HIF-1 α	Induce	Alleviate	Induce ferroptotic events via HIF-1 α /SLC7A11 pathway	HSC	Murine model of CCl ₄ -LF	[71]
IRP2	Induce	Alleviate	Promote iron accumulation	HSC	Murine model of CCl ₄ -LF	[65]
Transferrin	Inhibit	Alleviate	Buffer ferric iron	Hepatocyte	Murine model of CCl ₄ -LF or high dietary iron-induced LF	[10]
FGF21	Inhibit	Alleviate	HO-1 inhibition and Nrf2 activation	Hepatocyte	Murine model of iron overload-induced LF	[61]
Renal fibrosis						
IL-6	Inhibit	Alleviate	Downregulate the pro-fibrotic proteins, alleviate the activations and differentiations of macrophage F4/80+ cells	NA	Murine model of UUO-RF	[83]
HIF-1 α	Inhibit	Alleviate	Activates Akt/GSK-3 β -mediated Nrf2 nuclear translocation	NA	Murine model of folic acid-induced kidney injury	[79]
Smad3	Inhibit	Alleviate	Inhibit Smad3 phosphorylation and the transcription and protein level of NOX4	TEC	Murine model of UUO-RF	[84]

(Continued)

Table 2 (Continued).

Molecules	Function for Ferroptosis	Effect for Disease	Mechanisms	Test Cells	Disease Model	Reference
Cardiac fibrosis						
SGLT-2	Inhibit	Alleviate	Through the involvement of NLRP3 and MyD88-related pathways	Cardiomyocyte	Murine model of DIC	[102]
AMPK signaling	Inhibit	Alleviate	Regulate abnormal fatty acid metabolism and maintain mitochondrial function	Cardiomyocyte	Murine model of DIC	[104]
SIRT1	Inhibit	Alleviate	Activate SIRT1/Nrf2 signaling	Cardiomyocyte	Murine model of DIC	[103]
MLK3	Induce	Promote	Regulate the JNK/p53 signaling pathway-mediated oxidative stress	Cardiomyocyte	Murine model of TAC-CF	[12]
miR-224-5p	Induce	Promote	Downregulate FTH1	NA	Murine model of TAC-CF	[89]
Elabela	Inhibit	Alleviate	Modulate the IL-6/STAT3/GPX4 signaling	CMVEC	Murine model of Ang II-induced CF	[91]
xCT	Inhibit	Alleviate	Dispose ROS	Cardiomyocyte	Murine model of Ang II-induced CF	[92]
Pulmonary fibrosis						
miR-150-5p	Induce	Promote	Promotes lung fibroblast-to-myofibroblast transition and ferroptosis through the lncRNA ZFAS1/miR-150-5p/SLC38A1 axis	Fibroblast	Rat model of BLM-PL	[121]
SETDB1	Inhibit	Alleviate	Regulate Snail1 epigenetically; drive EMT gene reprogramming and ferroptosis	AEC	Rat model of BLM-PL	[118]

Abbreviations: HO-1, heme oxygenase-1; HSC, hepatic stellate cell; CCl4-LF, Carbon tetrachloride-induced liver fibrosis; BDL-LF, bile duct ligation-induced liver fibrosis; BRD7, bromodomain-containing protein 7; TRIM26, Tripartite motif-containing protein 26; SLC7A11, solute carrier family-7 member-11; m6A, N6-methyladenosine; NCOA4, nuclear receptor coactivator 4; HIF-1 α , hypoxia-inducible factor 1 α ; IRP2, Iron Regulatory Protein 2; FGF21, fibroblast growth factor 21; Nrf2, nuclear factor erythroid 2; IL-6, interleukin-6; UO-RF, unilateral ureteral obstruction-induced renal fibrosis; TEC, tubular epithelial cell; NOX4, NADPH oxidase 4; SGLT-2, sodium glucose co-transporter 2; DIC, doxorubicin-induced cardiomyopathy; MLK3, mixed lineage kinase 3; TAC-CF, transverse aortic constriction-induced cardiac fibrosis; FTH1, human ferritin heavy chain 1; STAT3, signal transducer and activator of transcription 3; GPX4, glutathione peroxidase 4; CMVEC, cardiac microvascular endothelial cell; CF, cardiac fibrosis; xCT, cystine/glutamate antiporter system; ROS, reactive oxygen species; SIRT1, sirtuin1; BLM-PL, bleomycin-induced pulmonary fibrosis; SETDB1, SET domain bifurcated histone lysine methyltransferase 1; EMT, epithelial mesenchymal transition; AEC, alveolar epithelial cell; NA, not applicable.

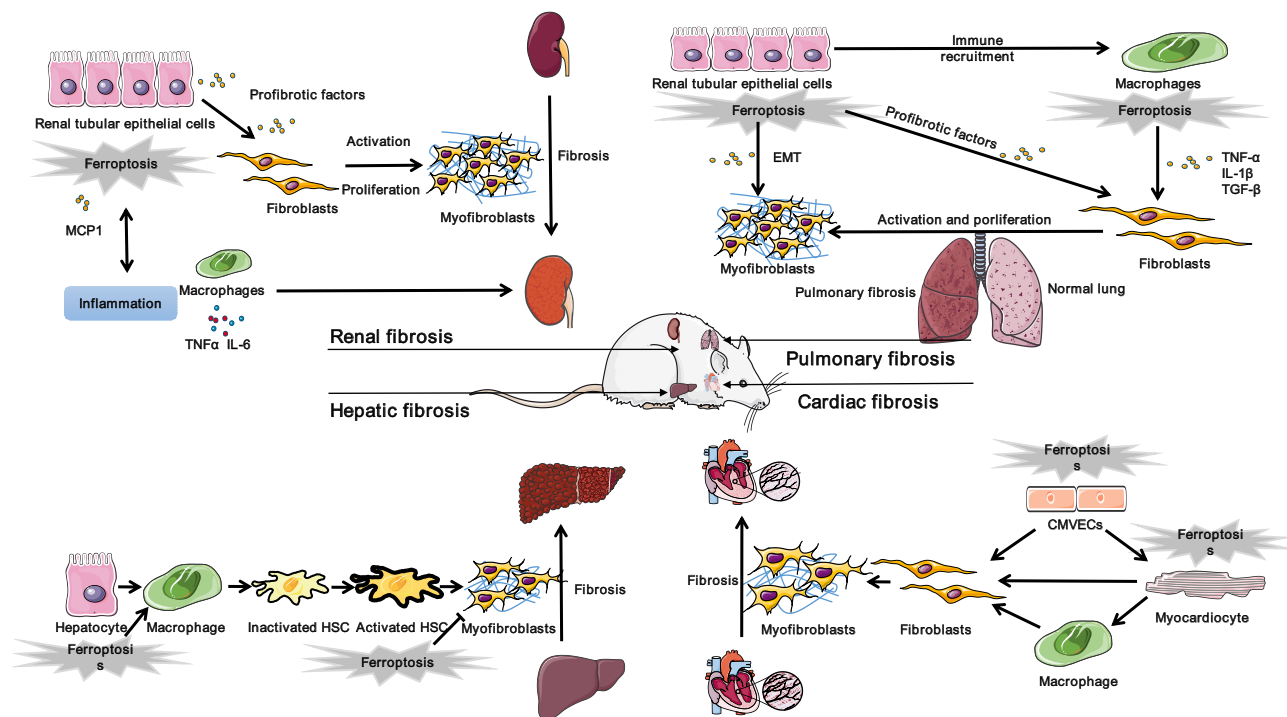


Figure 4 Ferroptosis in fibrotic therapy.

Abbreviations: TNF α , tumor necrosis factor α ; IL-6, interleukin-6; TGF- β , transforming growth factor- β ; MCP1, Monocyte chemoattractant protein-1; EMT, epithelial mesenchymal transition; HSC, hepatic stellate cell; CMVEC, cardiac microvascular endothelial cell.

of ELAVL1 via inhibiting the ubiquitin-proteasome pathway. The increased ELAVL1 binds to BECN1 mRNA and promotes BECN1/Beclin1 generation, thereby triggering autophagic ferritin degradation and finally inducing HSCs ferroptosis and alleviating liver fibrosis.⁶⁸ Similarly, ZFP36 destabilizes autophagy related 16 like 1 (ATG16L1) mRNA through binding to the AREs within the 3'-UTR, thus inhibiting macroautophagy/autophagy activation and mediating ferroptosis resistance. Ferroptosis-inducing compounds downregulate ZFP36 protein expression by the ubiquitin ligase F-box and WD repeat domain containing 7 (FBXW7/CDC4) which recognizes the SFSGLPS motif. Therefore, treatment erastin and sorafenib ameliorate liver fibrosis by inducing ZFP36 downregulation, ferritinophagy activation, and ferroptosis in HSCs.⁶⁹ Another study reported that ferroptosis inducers increased BRD7 protein expression via the inhibition of the ubiquitin-proteasome pathway. BRD7 directly binds with the p53 N-terminal transactivation domain, thereby promoting p53 mitochondrial translocation, which subsequently elevated the activity of solute carrier family 25 member 28 (SLC25A28) that resulted in excessive deposition of redox-active iron and hyperfunction of electron transfer chain. Collectively, Erastin or sorafenib treatment has been shown to suppress murine liver fibrosis by inducing HSCs ferroptosis via BRD7-P53-SLC25A28 axis.⁷⁰ In addition, sorafenib was also reported to trigger HSC ferroptosis via hypoxia-inducible factor (HIF)-1 α /SLC7A11 signaling, which attenuates liver injury and fibrosis in a mouse model of liver fibrosis induced by CCl₄.⁷¹ An E3 ubiquitin ligament TRIM26 was decreased in fibrotic liver tissues, which was originally identified as a tumor suppressor in hepatocellular carcinoma. A recent study found that TRIM26 promotes HSCs ferroptosis to mitigate liver fibrosis through mediating the ubiquitination of SLC7A11, the subunit in xCT system for lipid ROS scavenging.⁷² By using RNA sequencing analysis, Shen et al⁷³ found that N6-methyladenosine (m6A) modification also enhances HSC ferroptosis, possibly by activating autophagy via stabilizing BECN1 mRNA. Berberine (BBR) has been explored as a potential anti-liver fibrosis agent, Yi et al⁷⁴ reported that BBR attenuated liver fibrosis by promoting Fe²⁺ redox to activate ROS-mediated HSC ferroptosis. On the one hand, BBR suppressed the autophagy-lysosome pathway and increased cell ROS, which further accelerated the breakdown of the iron-storage protein ferritin and subsequent iron release from ferritin in HSCs; on the other hand, impaired autophagy enhanced BBR-mediated ferritin proteolysis to increase cellular Fe²⁺ overload via the ubiquitin-proteasome pathway in

Table 3 Drugs Targeting Ferroptosis in Fibrosis

Drugs	Targets	Function for Ferroptosis	Effect for Disease	Mechanisms	Test Cells	Disease Model	Reference
Liver fibrosis							
MgIG	HO-1	Induce	Alleviate	Promote the accumulation of iron and lipid peroxides	HSC	Rat model of CCl ₄ -LF	[62]
Artesunate	NA	Induce	Alleviate	Trigger ferritinophagy	HSC	Murine model of CCl ₄ -LF	[63]
Artemether	p53	Induce	Alleviate	p53-dependent induction of ferroptosis	HSC	Murine model of CCl ₄ -LF	[64]
Artemether	IRP2	Induce	Alleviate	Promote iron accumulation	HSC	Murine model of CCl ₄ -LF	[65]
Erastin or Sorafenib	ELAVL1	Induce	Alleviate	Promote autophagic ferritin degradation	HSC	Murine model of BDL-LF	[68]
Sorafenib; Erastin, or RSL3	ZFP36	Induce	Alleviate	ZFP36 downregulation, ferritinophagy activation, and ferroptosis induction	HSC	Murine model of BDL-LF	[69]
Sorafenib	HIF-1 α	Induce	Alleviate	Induce ferroptotic events via HIF-1 α /SLC7A11 pathway	HSC	Murine model of CCl ₄ -LF	[71]
Erastin or Sorafenib	BRD7-p53-SLC25A28 axis	Induce	Alleviate	Promote p53 mitochondrial translocation	HSC	Murine model of BDL-LF	[70]
Chrysophanol	NA	Induce	Alleviate	Lipid ROS accumulation	HSC	NA	[76]
Dihydroartemisinin	NCOA4	Induce	Alleviate	Autophagy activation and upregulation of NCOA4	HSC	Rat model of CCl ₄ -LF	[66]
Berberine	NA	Induce	Alleviate	Impaired the autophagy-lysosome pathway and increase cell ROS production	HSC	Murine model of TAA-LF and CCl ₄ -LF	[74]
Wild bitter melon	NA	Induce	Alleviate	ROS accumulation	HSC	NA	[67]
Recombinant FGF21	HO-1	Inhibit	Alleviate	HO-1 inhibition and Nrf2 activation	Hepatocytes	Murine model of iron overload-induced LF	[61]
Renal fibrosis							
Deferoxamine	Iron metabolism	Inhibit	Alleviate	Decrease iron deposition; inhibit TEC ferroptosis; reduce MCP-1 secretion and macrophage chemotaxis	TEC	Murine model of UUO-RF or IRI-RF; rat model of 5/6 nephrectomy-induced CKD	[81,82]
Liproxstatin-I	Lipid peroxidation	Inhibit	Alleviate	Inhibit ferroptosis-mediated TECs death	TEC	Murine model of UUO-RF	[11]
Ferrostatin-I	Lipid peroxidation	Inhibit	Alleviate	Induce ferroptotic events via HIF-1 α /HO-1 pathway	NA	Murine model of diabetic renal tubular injury	[78]
Tocilizumab mimotope vaccination	IL-6	Inhibit	Alleviate	Down-regulate the pro-fibrotic proteins; inhibits ferroptosis; alleviate the activation and differentiation of macrophage F4/80+ cells	NA	Murine model of UUO-RF	[83]

Roxadustat	HIF-1 α	Inhibit	Alleviate	Activates Akt/GSK-3 β -mediated Nrf2 nuclear translocation	NA	Murine model of folic acid-induced kidney injury	[79]
Tectorigenin	Smad3	Inhibit	Alleviate	Inhibite Smad3 phosphorylation and the transcription and protein level of NOX4	TEC	Murine model of UUO-RF	[84]
Nobiletin	NA	Inhibit	Alleviate	Mitigate oxidative stress; attenuate EMT; alleviate leucocyte cell infiltration	NA	Murine model of UUO-RF	[85]
Cardiac fibrosis							
Doxorubicin	Fe ²⁺	Induce	Promote	Downregulate GPX4 and induces excessive lipid peroxidation	Cardiomyocyte	Murine model of DIC	[101]
Empagliflozin	SGLT-2	Inhibit	Alleviate	Through the involvement of NLRP3 and MyD88-related pathways	Cardiomyocyte	Murine model of DIC	[102]
Fisetin	SIRT1/Nrf2 signaling	Inhibit	Alleviate	Activates SIRT1/Nrf2 signaling pathway	Cardiomyocyte	Rat model of DIC	[103]
Salidroside	AMPK signaling	Inhibit	Alleviate	Regulate abnormal fatty acid metabolism and maintain mitochondrial function	Cardiomyocyte	Murine model of DIC	[104]
Histochrome	Nrf2	Inhibit	Alleviate	Decrease ROS level; increase GPX4 and free GSH levels	Cardiomyocyte	Rat model of MIRI	[100]
Etomidate	Nrf2	Inhibit	Alleviate	Activate Nrf2 pathway	NA	Rat model of MIRI	[98]
Dexmedetomidine	SLC7A11/GPX4 axis	Inhibit	Alleviate	Activate SLC7A11/GPX4 axis	NA	Rat model of MIRI	[99]
Astragaloside IV	Nrf2	Inhibit	Alleviate	Suppressing oxidative stress; enhance Nrf2 signaling	NA	Murine model of AIC	[105]
Pulmonary Fibrosis							
Deferoxamine	Iron metabolism	Inhibit	Alleviate	Iron deposition and mitochondrial dysfunction	Alveolar type II cells; bronchial epithelial cells	Murine model of BLM-PF	[13,112,116]
Liproxstatin-I	Lipid peroxidation	Inhibit	Alleviate	Down-regulation of TGF- β 1 by the activation of Nrf2 pathway	NA	Murine model of radiation-induced PF	[127]
Ferrostatin-I	Lipid peroxidation	Inhibit	Alleviate	Inhibit lipid peroxidation	Macrophage	Murine model of SiO ₂ -induced PF	[122]

Abbreviations: MgIG, Magnesium isoglycyrrhizinate; HO-1, heme oxygenase-1; HSC, hepatic stellate cell; CCl₄-LF, Carbon tetrachloride-induced liver fibrosis; IRP2, Iron Regulatory Protein 2; BDL-LF, bile duct ligation-induced liver fibrosis; HIF-1 α , hypoxia-inducible factor 1 α ; SLC7A11, solute carrier family-7 member-11; BRD7, bromodomain-containing protein 7; ROS, reactive oxygen species; NCOA4, nuclear receptor coactivator 4; TAA-LF, thioacetamide-induced liver fibrosis; FGF21, fibroblast growth factor 21; Nrf2, nuclear factor erythroid 2; TEC, tubular epithelial cell; MCP-1, monocyte chemoattractant protein 1; UUO-RF, unilateral ureteral obstruction-induced renal fibrosis; IRI-RF, ischemia/reperfusion injury-induced renal fibrosis; CKD, chronic kidney disease; IL-6, interleukin-6; NOX4, NADPH oxidase 4; EMT, epithelial mesenchymal transition; GPX4, glutathione peroxidase 4; DIC, doxorubicin-induced cardiomyopathy; SGLT-2, sodium glucose co-transporter 2; SIRT1, sirtuin1; GSH, glutathione; MIRI, myocardial ischemia-reperfusion injury; AIC, Adriamycin-induced cardiomyopathy; BLM-PF, bleomycin-induced pulmonary fibrosis; PF, pulmonary fibrosis; NA, not applicable.

HSCs. Other studies discovered that exosomal miR-222 derived from hepatitis B virus-infected hepatocytes promote HSCs activation through inhibiting TERC-induced ferroptosis.⁷⁵ Chrysophanol, a natural plant composition, alleviates hepatitis B virus X protein-induced HSC activation and liver fibrosis by regulating ferroptosis.⁷⁶ Collectively, these findings will provide a new perspective to understand the mechanism of ferroptosis and find effective treatment in liver fibrosis.

Ferroptosis and Renal Fibrosis

Renal fibrosis refers to excessive deposition of the fibrotic matrix within the parenchyma during chronic kidney injury, which represents the common final pathway of nearly all chronic and progressive nephropathies.⁷⁷ Ferroptosis has been implicated in the development of renal fibrosis in distinct experimental animal models such as diabetic nephropathy,⁷⁸ high fat diet-induced renal injury,⁶⁰ folic acid-induced kidney injury,⁷⁹ unilateral ureter obstruction (UUO),¹¹ ischemia/reperfusion injury (IRI),⁸⁰ and 5/6 nephrectomy-induced chronic kidney disease (CKD).⁸¹ Renal tubular epithelial cells (TECs) are one of the most vulnerable cell types to ferroptotic stress.¹¹ A recent study reported that ureteral obstruction leads to ferroptosis in renal TECs, which are essential for UUO-induced renal fibrosis. Specifically, ferroptotic cell death in TECs triggers the secretion of profibrotic mediators such as TGF- β , CTGF, and PDGF, which subsequently regulate the proliferation and differentiation of interstitial fibroblasts in a paracrine fashion. The ferroptosis inhibitor liproxstatin-1 (Lip-1) alleviated ferroptosis-induced renal fibrosis by restraining ferroptosis-mediated TECs death and lessening the activation of surrounding fibroblasts via inhibiting the paracrine activity of profibrotic factors of epithelial cells.¹¹ Another study of a mouse model with UUO or IRI demonstrated that TECs ferroptosis facilitates monocyte chemotactic protein 1 secretion and macrophage chemotaxis, thus promoting interstitial fibrosis, which can be largely mitigated by ferroptosis inhibitor Fer-1 or DFO.⁸² In addition to triggering cell death of TECs, ferroptotic stress may also enhance the damage-associated state of proximal tubular cells, which leads to persistent inflammation and fibrosis during failed renal repair after severe injury and the acute kidney injury-to-CKD transition.⁸⁰ Consequently, experimental reagents and traditional Chinese medicine as well as its effective components targeting ferroptosis show strong therapeutic potential for renal fibrosis in preclinical studies. In a 5/6 nephrectomy-induced CKD rat model, renal interstitial fibrosis is exacerbated by ferroptosis inducer cisplatin, while ferroptosis inhibitor showed antifibrotic effects by regulating the TGF- β 1/Smad3 signaling pathway.⁸¹ Tocilizumab, a humanized monoclonal antibody targeting interleukin-6 (IL-6) signaling, ameliorates the renal fibrosis and reduces ferroptosis simultaneously in the UUO model.⁸³ However, in this study the internal relationship of ferroptosis and fibrosis requires further exploration. Tectorigenin, a small molecule derived from the medicinal herbal *Belamcanda chinensis*, has proved to possess protective activities against ferroptosis and fibrosis both in vivo and in vitro, which is mediated by Smad3-induced expression of NADPH oxidase 4 (NOX4).⁸⁴ Nobiletin, a flavonoid in the peel of citrus fruits, ameliorates ferroptosis-associated injury and renal fibrosis in the kidney of UUO mice, suggesting that Nob treatment has anti-ferroptosis and antifibrotic effects.⁸⁵ Pretreatment of FG-4592, an inhibitor of prolyl hydroxylase of HIF, plays a protective role in the early stage of folic acid-induced kidney injury and retards the progression of renal fibrosis, mainly by alleviating ferroptosis via stabilizing HIF-1 α and Akt/GSK-3 β -mediated Nrf2 activation.⁷⁹ Interestingly, the HIF-1 α /HO-1 pathway was also reported to take part in aggravation of tubular injury and fibrosis by ferroptosis in the kidneys of diabetic mouse models.⁷⁸ However, the exact mechanisms of these reagents and drugs in induction of ferroptosis and treatment of fibrosis are not fully elucidated. Therefore, in-depth exploration with a genetic model are demanding areas for future research to advance the field.

Ferroptosis and Myocardial Fibrosis

Myocardial fibrosis includes reactive fibrosis and replacement fibrosis. While the former occurs in perivascular spaces and corresponds to similar fibrogenic responses in other tissues, the latter occurs at the site of myocyte loss.⁸⁶ Myofibroblasts in the injured heart are largely derived from resident fibroblasts, which are the most abundant cells in the myocardium. In response to injury, cardiac fibroblasts proliferate and differentiate into myofibroblasts under the mediation of classic factors such as TGF- β , endothelin-1, and angiotensin-1.⁸⁷ Myocardial infarction (induced by permanent occlusion of the left anterior descending coronary artery) and pressure overload-induced cardiac hypertrophy (triggered by transverse aortic constriction) are two common experimental models for cardiac fibrosis. Cardiac fibrosis

has deleterious effects on both myocardial contractility and myocardial electrophysiology, resulting in a growing incidence of arrhythmia and sudden cardiac death. Cardiac fibrosis is thought to play crucial roles in cardiac remodeling, which is closely associated with heart failure.⁸⁸ Moreover, ferroptosis is enhanced in heart failure. Myocardium in murine model of heart failure showed apparent fibrosis as well as accumulation of iron and malondialdehyde,⁸⁹ and there is evidence that cell death mediated by ferroptosis combined with activated autophagy promotes heart failure progression through the TLR4-NOX4 pathway.⁹⁰ During the advanced stages of chronic heart failure, ferroptosis induced by Mixed lineage kinase 3 (MLK3) via JNK/p53 signaling pathway in cardiomyocytes mediates myocardial fibrosis. MiR-351 negatively regulated the expression of MLK3, thereby exerting a protective effect on ventricular remodeling in heart failure caused by pressure overload.¹² In another study of myocardial fibrosis in response to pressure overload, elabela and Fer-1 alleviate Ang II-mediated ferroptosis of cardiac microvascular endothelial cells as well as subsequent myocardial fibrosis through modulating the IL-6/STAT3/GPX4 signaling pathway.⁹¹ SLC7A11 gene-encoded plasma membrane xCT is identified as a cardioprotective factor in cardiac hypertrophic diseases, since xCT alleviates angiotensin II-induced cardiac fibrosis and pathological cardiac remodeling by inhibiting ferroptosis.⁹²

Many studies have shown that ferroptosis occurs in myocardial infarction, myocardial IRI, and heart failure, all of which can lead to the formation of fibrosis.⁸⁷ GPX4 is significantly downregulated at the transcriptional level in the early and middle stages of myocardial infarction, which results in the accumulation of lipid peroxide and subsequently ferroptosis in cardiomyocytes.⁹³ Similarly, the Nrf2/Hmox1 pathway involves in the iron excess which leads to ferroptotic cell death during early and middle stages of MI.⁹⁴ Lip-1 protects myocardium against IRI by inhibiting ferroptosis through increasing GPX4 and decreasing ROS.⁹⁵ Fer-1, another ferroptosis inhibitor, alleviates myocardial damage during hypoxia/reoxygenation by mitigating ER stress in diabetes myocardial IRI.⁹⁶ Rapamycin reduces IRI by suppressing ferroptosis via regulating iron transportation and controlling iron metabolism in cardiomyocytes.⁹⁷ Accordingly, drugs targeting ferroptosis showed antifibrosis effects in animal experiments of myocardial ischemia-reperfusion injury. Etomidate, a short acting anesthetic, alleviated IRI-induced myocardial fibrosis by inhibiting ferroptosis through Nrf2 pathway, which can be eliminated by erastin.⁹⁸ Another anesthetic, dexmedetomidine, has also been shown to possess a protective role in IRI-induced myocardial injury and fibrosis by inhibiting ferroptosis via enhancing the expression of SLC7A11 and GPX4.⁹⁹ A study of IRI also reported that intravenous injection of histochrome significantly mitigates cardiac fibrosis. Mechanistically, histochrome treatment downregulates intracellular and mitochondrial ROS levels by activating the Nrf2 pathway. In vitro experiment indicated that histochrome also inhibited erastin- and RSL3-induced ferroptosis in rat neonatal cardiomyocytes by maintaining the intracellular glutathione level and through upregulated activity of GPX4.¹⁰⁰ In summary, ferroptosis participants in the pathogenesis of multiple fibrosis-related cardiovascular diseases, targeting ferroptosis may be an effective therapeutic strategy for these diseases, thereby reducing the formation of myocardial fibrosis.

Interstitial fibrosis also occurs in doxorubicin (DOX)-induced cardiomyopathy (DIC) and adriamycin (ADR)-induced cardiomyopathy. Ferroptosis has been proven to contribute to play a key role in progression of DIC and it has been reported that ferroptosis is the main form of RCD in DOX cardiotoxicity. Specifically, DOX induces mitochondria-dependent ferroptosis by decreasing GPX4 and promoting excessive LPO through DOX-Fe²⁺ complex in mitochondria.¹⁰¹ The selective inhibitor of the sodium glucose co-transporter 2 empagliflozin reduced ferroptosis and fibrosis in doxorubicin-treated mice through the involvement of NLRP3 and MyD88-related pathways.¹⁰² Fisetin, a natural flavonoid with cardioprotective effects, markedly ameliorates myocardial fibrosis and ferroptosis of cardiomyocytes in the DOX-induced cardiomyopathy rat and H9c2 cell models. Further studies showed that fisetin protects against DOX-induced cardiomyopathy by inhibiting ferroptosis via SIRT1/Nrf2 signaling pathway activation.¹⁰³ Salidroside, an extraction from traditional Chinese medicine *Rhodiola rosea*, also significantly attenuated ferroptosis and fibrosis in a mice model of DIC. However, the molecular mechanism is different; the anti-ferroptotic effects of salidroside are mediated by activating AMPK-dependent signaling pathways including regulating abnormal fatty acid metabolism and maintaining mitochondrial function.¹⁰⁴ In addition, Astragaloside IV, an active ingredient isolated from traditional Chinese medicine *Astragalus membranaceus*, exerts protective effects against adriamycin-induced myocardial fibrosis, which may partly attribute to its anti-ferroptotic effect via Nrf2 signaling.¹⁰⁵ Overall, targeting ferroptosis may represent a novel treatment that improves cardiac fibrosis from drug-induced cardiomyopathy.

Ferroptosis and Pulmonary Fibrosis

The etiology of lung fibrosis is diverse. In addition to infections, toxics, and radiation, scleroderma and sarcoidosis can also give rise to lung fibrosis.¹⁰⁶ Intratracheal administration of bleomycin (BLM) is the most frequently used experimental animal model of pulmonary fibrosis. Histopathologically, pulmonary fibrosis is characterized by destruction of the alveolar structure, lung fibroblast proliferation, and ECM deposition.¹⁰⁷ Pulmonary fibrosis reduces lung compliance, disturbs gas exchange, and restricts lung function.¹⁰⁸ As the disease continues to advance, pulmonary hypertension, right-sided heart failure, respiratory failure, and even death occur.¹⁰⁸

Idiopathic pulmonary fibrosis (IPF), a rapidly progressive and usually fatal lung disease without clear cause and effective therapy, is the most pernicious of pulmonary fibrosis.^{109–111} It is now well-established that ferroptosis plays a key role in the pathogenesis of pulmonary fibrosis. Iron accumulation substantially increased both in lung tissue sections from patients with IPF and in experimental bleomycin-induced pulmonary fibrosis, which corresponded with the pulmonary fibrosis and a decline in lung function.^{13,112} Bioinformatics analysis also supported that ferroptosis-related genes are significantly correlated with the development of IPF.^{113–115} Moreover, some ferroptosis-related genes in the bronchoalveolar lavage showed prognostic value in patients with IPF.^{113,115} Current studies mainly focus on the role of ferroptosis in the alveolar epithelial injury, fibroblast-to-myofibroblast transition, and epithelial-mesenchymal transition (EMT), all of which are important biological processes in the development of IPF. It is now established that dysfunction of alveolar epithelial cells, especially alveolar type 2 (AT II) cells, drive the pathogenesis of IPF.¹³ Correspondingly, pulmonary iron overload leads to ferroptosis of AT II cells and pulmonary fibrosis, and ferroptosis inhibitor DFO completely reverses the pro-fibrosis phenotype and mitigates mitochondrial damage induced by BLM via decreasing iron deposition and ferroptosis in AT II cells.¹³ In another study, exogenous administration of a water-soluble iron salt, Fe³⁺ ammonium citrate in human lung fibroblasts can increase cellular proliferation, enhance the expression of ECM-related genes, resulting in the development of IPF.¹¹⁶ DFO has also been reported to inhibit TGF- β -induced EMT, thus ameliorating BLM-induced IPF.¹¹² In line with this finding, ferroptosis inducer erastin promotes EMT in a mouse lung epithelial cell line, which is accompanied by reactive oxygen species (ROS) production and upregulated expression of heme oxygenase-1.¹¹⁷ Histone methyltransferase SET domain bifurcated 1 (SETDB1) is decreased in TGF- β -induced EMT, which plays an antifibrotic role in pulmonary fibrosis. Knockdown of SETDB1 enhances TGF- β -induced EMT and ferroptosis in human alveolar epithelial cells, as decreased SETDB1 reduces the histone H3 lysine 9 trimethylation of Snail and enhances the occurrence of TGF- β -induced EMT, which further increased lipid ROS and Fe²⁺ ions.¹¹⁸ Therefore, ferroptosis occurs during EMT induced by TGF- β ; however, the mechanism underlying the relationship between ferroptosis and EMT warrants further study.¹¹⁹ Other studies investigating the relationship of ferroptosis and fibroblast-to-myofibroblast transition in fibrosis reported that Erastin promoted fibroblast-to-myofibroblast differentiation via increasing LPO and inhibiting the expression of GPX4, which can be blocked by Fer-1.¹²⁰ Upregulation of lncRNA zinc finger antisense 1 (lncRNA ZFAS1) was observed in BLM-induced PF rat lung tissue and in TGF- β 1-induced human fetal lung fibroblast cells. As a competing endogenous RNA, ZFAS1 was demonstrated to promote fibroblast activation and LPO by sponging miR-150-5p and downregulating SLC38A1 expression, suggesting the role of lncRNA ZFAS1/miR-150-5p/SLC38A1 axis in the progression of PF.¹²¹ Apart from epithelial cells and fibroblast, ferroptosis in macrophages also promotes the development of IPF. Liu et al¹²² demonstrated that silicosis induces ferroptosis in macrophages, which leads to the secretion of pro-fibrotic cytokines and fibrosis. In summary, these preliminary explorations provide evidences of iron deposition and ferroptosis in alveolar macrophages, AT II cells and fibroblasts contribute to the development and progression of IPF.

Radiation-induced lung fibrosis (RILF) is a category of radiation-induced lung injury (RILI), which is a common and severe complication of thoracic radiotherapy.¹²³ It is now believed that both direct radiation damage to DNA and radiation-enhanced production of ROS cause cellular damage in RILF.^{124–126} In addition, ROS caused by irradiation is closely related to inflammation and fibrosis.^{125,126} Apart from apoptosis, several lines of evidence suggested that ferroptosis might play a critical role in the process of RILF. Firstly, the expression level of GPX4 is obviously decreased in RILF mice.¹²⁷ Secondly, ROS levels are significantly upregulated in acute RILI.¹²⁸ Thirdly, ferroptosis inhibitor Lip-1 inhibited the collagen deposition and reduced hydroxyproline in the RILF model.¹²⁷ Fourthly, the pathological changes in

RILI are substantially alleviated by ferroptosis inhibitors.¹²⁸ Finally, studies of Li et al¹²⁷ demonstrated that ferroptosis inhibitor Lip-1 mitigated RILF through downregulation of TGF- β 1 via the activating Nrf2 pathway.

Paraquat (PQ), an efficacious and widely-used herbicide, is considered as one of the most frequently-used and incurable poisoning substances for suicide.¹²⁹ Exposure to PQ causes severe damage in the lungs and eventually leads to pulmonary fibrosis.¹²⁹ Generally, the toxicity of PQ is the result of formation of high energy oxygen free radicals and peroxidation of unsaturated lipids in the cell.¹²⁹ Ferroptosis inhibitors with antioxidant properties appear to be one of the main mechanisms in PQ detoxification.¹³⁰ For example, iron chelators DFO prevent PQ toxicity via inhibition of hydroxyl radical production and inhibition of PQ uptake by AT II cells.^{129,131} While vitamin E serves as a lipophilic antioxidant to inhibit LPO in ferroptosis, vitamin E deficiency increases acute PQ toxicity and deterioration of histological lung damage in animals, with a decreased survival rate.¹²⁹ Edaravone protects the lung against PQ toxicity by alleviating oxidative stress and inflammatory responses.¹³² In the same year, it was reported that edaravone suppresses ferroptosis by scavenging free radicals and lessening oxidative stress.¹³³ Ebselen is an inhibitor of apoptosis and ferroptosis which also has anti-inflammatory and cytoprotective effects.¹³³ As ebselen efficiently scavenges ROS and RNS, it also exhibits antioxidant activity.^{134,135} More than 30 years ago, it was found that ebselen combined with N-acetylcysteine can be used to treat PQ poisoning. Mechanistically, ebselen might confer resistance to ferroptosis, possibly by inhibiting the activity of glutaminase and NADPH-oxidase and suppressing inflammasome mediated inflammatory pathways.⁹¹ Thiazolidinedione alleviates the PQ toxicity by inhibiting ferroptosis via decreasing ACSL4 activity and increasing ROS scavenging enzymatic activity.¹³⁶ Together these findings raise the possibility of targeting ferroptosis for the treatment of PQ poisoning.

Concluding Remarks

Novel therapeutic strategies are in urgent demand for fibrotic diseases. A growing number of studies have provided insights into the mechanisms and factors associated with the regulation of fibrosis by ferroptosis. New techniques like single-cell multi-omics and genetic approaches makes it possible to explore mechanisms of ferroptosis and fibrosis at the single-cell level with unexampled resolution.⁸⁰ In this context, the development of antibodies and drugs that modulate ferroptosis via both its induction and its inhibition holds great potential for the treatment of patients with a broad spectrum of fibrotic diseases, as the ultimate goal of understanding the role of ferroptosis in fibrosis is to leverage this relationship for the therapeutic benefit of patients suffering from fibrotic diseases. Research in this field is still in its infancy, and much work is needed to elucidate the detailed molecular mechanisms and regulatory networks through which ferroptosis participates in fibrotic diseases. Besides, a translational gap remains where we can convert the putative targets into effective clinical drugs for ferroptosis-based disease therapy.¹³⁷ Targeting ferroptosis may unleash unprecedented opportunities for pharmacological intervention. Future work is expected to provide novel therapeutic strategies for preventing, controlling, and treating fibrosis.

Abbreviations

ECM, extracellular matrix; RCD, regulated cell death; GPX4, glutathione peroxidase 4; LPO, lipid peroxidation; PLOOHs, phospholipid hydroperoxides; LOXs, lipoxygenases; POR, cytochrome P450 oxidoreductase; ROS, reactive oxygen species; LIP, labile iron pool; Fe³⁺, ferric; Trf, transferrin; TFRC, transferrin receptor; Fe²⁺, ferrous; FHC, ferritin heavy chain; PUFA-PLs, phospholipids containing polyunsaturated fatty acid chains; PUFAs, polyunsaturated fatty acids; ACSL4, Acyl-CoA synthetase long-chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; GSH, glutathione; FSP1, ferroptosis suppressor protein 1; CoQ10, coenzyme Q10, ubiquinol; DHODH, dihydroorotate dehydrogenase; GCH1, GTP cyclohydrolase 1; BH4, tetrahydrobiopterin; DHFR, dihydrofolate reductase; SLC7A11, solute carrier family-7 member-11; TGF- β , transforming growth factor- β ; EMT, epithelial-mesenchymal transition; HSCs, hepatic stellate cells; Fer-1, ferrostatin-1; CCl₄, tetrachloride; HO-1, heme oxygenase-1; FGF21, Fibroblast growth factor 21; Nrf2, nuclear factor erythroid 2-related factor 2; DFO, deferoxamine; ART, Artemether; ELAVL1, ELAV like RNA binding protein 1; ZFP36, ZFP36 ring finger protein; BRD7, bromodomain-containing protein 7; TRIM26, tripartite motif-containing protein 26; AREs, AU-rich elements; 3'-UTR, 3rd-untranslated region; ATG16L1, autophagy related 16 like 1; FBXW7/CDC4, F-box and WD repeat domain containing 7; SLC25A28, solute carrier

family 25 member 28; HIF, hypoxia-inducible factor; BBR, Berberine; UUU, unilateral ureteral obstruction; IRI, ischemia/reperfusion injury; CKD, chronic kidney disease; TECs, tubular epithelial cells; Lip-1, liproxstatin-1; IL-6, interleukin-6; NOX4, NADPH oxidase 4; MLK3, Mixed lineage kinase 3; DOX, doxorubicin; DIC, doxorubicin-induced cardiomyopathy; ADR, adriamycin; BLM, bleomycin; AT II, alveolar type 2; ZFAS1, zinc finger antisense 1; RILF, radiation-induced lung fibrosis; RILI, radiation-induced lung injury; PQ, paraquat.

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The authors have declared conflicts of interest in relation to this work.

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