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Review

Lipid Peroxidation in Ferroptosis and Association with Nonalcoholic Fatty Liver Disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) constitutes a commonly diagnosed liver pathology with perturbed lipid metabolism, which is mainly caused by excessive accumulation of fat in hepatocytes by various pathogenic factors. Currently, there are no effective drug treatments for NAFLD. Ferroptosis represents a novel form of programmed cell death depending on iron, which is driven by large cellular amounts of reactive oxygen species (ROS) and lipid peroxides. Ferroptosis plays critical regulatory roles in the pathogenesis of NAFLD, and overaccumulation of Fe^{2+} contributes to lipid peroxidation, which subsequently aggravates NAFLD. Therefore, ferroptosis suppression might constitute an important target for NAFLD treatment. This article reviews the discovery, production pathways, and defense mechanisms of ferroptosis, and explores its association with NAFLD. This may provide new reference targets and strategies for the development of NAFLD drugs from the perspective of ferroptosis.

Keywords: ferroptosis; lipid peroxidation; nonalcoholic fatty liver disease; ferritinophagy

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) constitutes the commonest chronic liver disease, affecting about 25% of humans globally and projected to become the main reason for liver transplantation by 2030, therefore representing a worldwide public health issue [1–3]. Current evidence shows that NAFLD is a generic term designating a multisystem disease, including a range of liver diseases with metabolic risk factors [4,5]. NAFL refers to hepatic steatosis, in which fat is present in more than 5% of hepatocytes. Subsequently, nonalcoholic steatohepatitis (NASH) occurs after NAFL aggravates different injuries. NAFL/NASH is associated with hepatocellular damage and usually has four phases: (1) fat overaccumulation in hepatocytes, also termed NAFL; (2) hepatocyte ballooning degeneration and diffuse lobular inflammation; (3) fibrosis, caused by inflammation and hepatocellular damage, which features an excessive extracellular matrix (ECM) accumulation that alters the normal hepatocyte features; (4) cirrhosis that sustainably progresses to the terminal stage of liver disease. Approximately 20% of NAFL cases progress to NASH, and more than 40% of NASH cases develop fibrosis; liver transplantation is considered in case of severe cirrhosis [6,7].

Recently, ferroptosis has rapidly attracted attention among scientists studying chronic liver disease, as excessive accumulation of iron, which is the main feature of most liver diseases, can cause oxidative damage in the liver. Evidence suggests ferroptosis is tightly associated with lipid peroxidation (LPO) in NAFLD and can therefore be an important target for preventing and treating NAFLD [8]. This article reviews the generation and regulation of ferroptosis and elucidates the association of LPO in ferroptosis with NAFLD.

2. Ferroptosis

2.1 Development of Ferroptosis

Ferroptosis, a newly identified iron-dependent cell death, is characterized by iron accumulation and LPO, and has different morphological, biochemical and genetic characteristics from other forms of programmed cell death. Ultrastructural analysis showed the key morphological features distinguishing ferroptosis from apoptosis, necrosis and autophagy include cell membrane rupture and blister, decreased mitochondrial size relative to normal, mitochondrial ridge reduction or disappearance, and increased membrane density [9].

LPO is a key factor inducing ferroptosis, with polyunsaturated fatty acids (PUFAs) generally serving as reaction substrates. Carbon-carbon double bonds and carbon-hydrogen bonds in PUFAs are transformed to generate PUFAs-CoA by acyl-CoA synthetase long chain family member 4 (ACSL4), and PUFAs-coenzyme A (PUFAs-CoA) are then esterified by lysophosphatidylcholine acyltransferase 3 (LPCAT3) into substrate PUFAs-PL in membrane phospholipids. Therefore, ACSL4 is considered a ferroptosis marker [10]. Free radicals interact with the hydrogen of PUFAs-PL to form the phospholipid radical (PL·), which then reacts with oxygen to generate phospho-

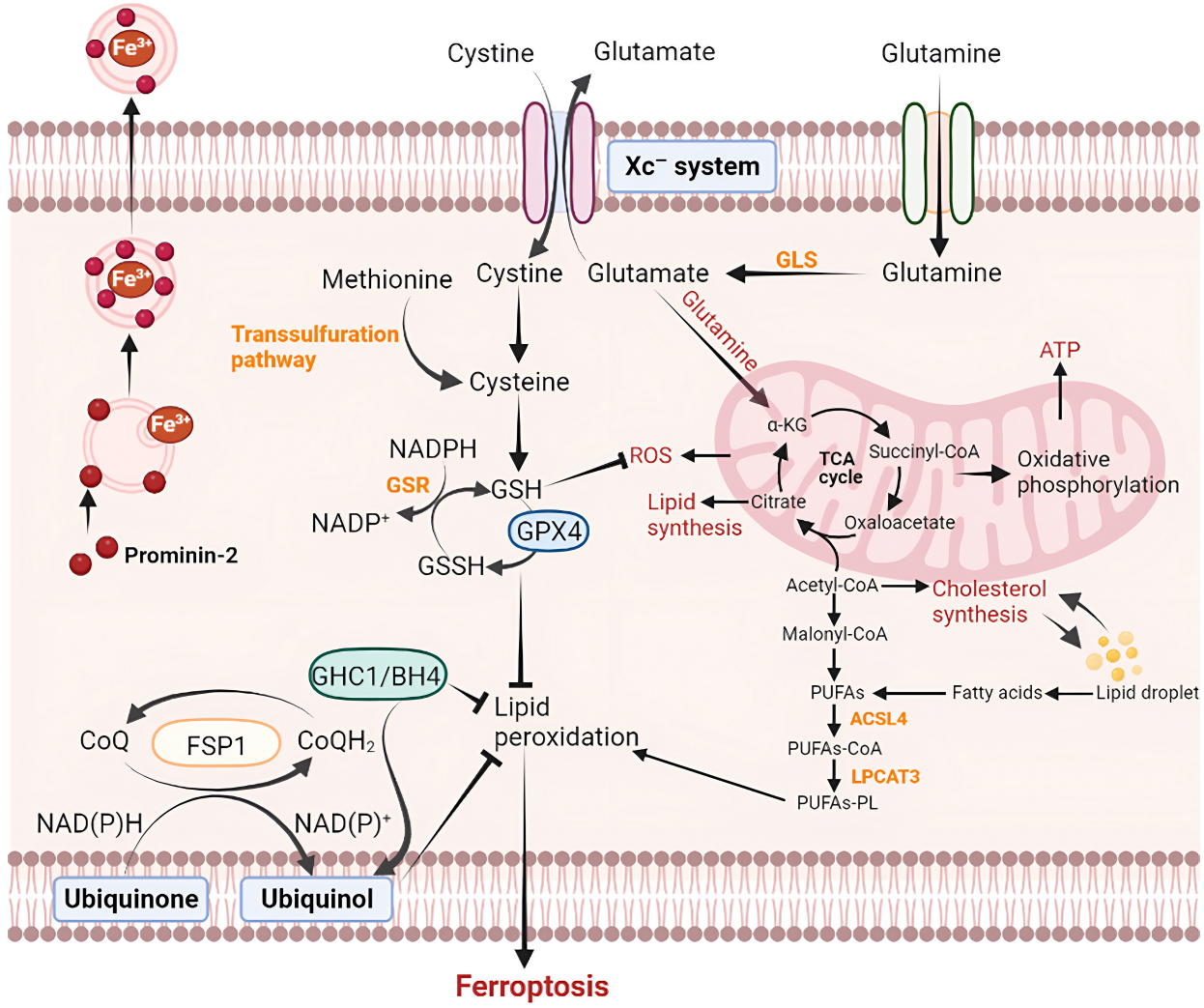

Fig. 1. Pathways of ferroptosis. Ferroptosis can be triggered through inhibiting the system Xc^-/GSH/GPX4 axis or the accumulation of Fe^{2+} and lipid peroxidation. Blocking or inhibiting the Xc^-/GSH/GPX4 axis can result in decreased intracellular cysteine levels and suppressing the lipid repair function of GPX4. Consequently, disruption of the antioxidant capacity of cells contributes to the initiation and progression of ferroptosis. In lipid metabolism, the most important characteristics of ferroptosis are the increase of intracellular iron ion concentration and the abnormal accumulation of lipid ROS. FSP1 can negatively regulate ferroptosis through CoQ10, thereby inhibiting the delivery of lipid peroxides. Ferritinophagy plays a crucial role in the association between ferritinophagy and ferroptosis. Intracellular ferritin is transported to autophagy lysosomes for degradation, leading to the release of free iron and ultimately triggering ferroptosis. Abbreviations: GPX4, glutathione peroxidase 4; PUFAs, polyunsaturated fatty acids; ACSL4, acyl-CoA synthetase long chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; PL-OO·, phospholipid peroxyl radical; TCA, tricarboxylic acid; GSH, glutathione; ROS, reactive oxygen species; FSP1, ferroptosis suppressor protein 1; CoQ10, coenzyme Q10; GCH1, GTP cyclohydrolase 1. ATP, adenosine triphosphate.

lipid peroxyl radical (PL-OO·). Hydrogen is extracted from other PUFAs to generate lipid peroxides (PL-OOH) and PL·, and so on. However, excessive Fe^{2+} catalyzes the reduction of PL-OOH to PL-O through the Fenton reaction, which further promotes non-enzymatic free radical chain reaction, ultimately altering cell membrane integrity and inducing ferroptosis [11].

LPO occurs in many subcellular structures such as mitochondria, endoplasmic reticulum (ER) and lysosomes [12]. Reactive oxygen species (ROS) generated by the tricarboxylic acid (TCA) cycle and electron transport chain (ETC) in the mitochondria, can target PUFAs-double bonds to produce LPO and induce ferroptosis [13]. Moreover, ER stress has a definite correlation with ferroptosis, and lysosomal ROS may also participate in ferroptosis as ferrostatin was observed in lysosomes [14,15]. However, the specific mechanism remains undefined. Reactive lipid species generated by LPO include 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA), whose contents are the highest and associated with ferroptosis [16].

2.2 Defense Mechanisms against Ferroptosis

Currently, three pathways are known to inhibit LPO to prevent ferroptosis, including the glutathione pathway (GSH-GPX4), the ubiquinone pathway (NADPH-FSP1-CoQ10) and the tetrahydrobiopterin pathway (GCH1-BH4) [17]. Firstly, the glutathione peroxidase 4 (GPX4) pathway is one of the commonest defense systems, which mainly depends on the Xc^- transporter system, glutathione (GSH) synthesis and GPX4 activity. The Xc^- system transports equal amounts of extracellular cystine and intracellular glutamic acid. Cystine then undergoes reduction by GSH to generate cysteine, thereby interfering with LPO reactions and preventing ferroptosis. Nuclear factor erythroid 2-related factor 2 (Nrf2) represents a major upstream regulatory gene of defense systems that can target the subunit solute carrier family 7 member 11 (SLC7A11), the light chain subunit of the Xc^- system [18]. Studies have suggested that drugs may activate Nrf2 to initiate the GPX4 defense mechanism to mediate ferroptosis and reduce the occurrence and development of diseases, especially NAFLD [19–21]. This provides a promising strategy for developing drug candidates targeting Nrf2 for the treatment of NAFLD.

Secondly, ferroptosis suppressor protein 1 (FSP1) is a newly reported strong and effective inhibitor of ferroptosis. The FSP1 pathway does not depend on GSH for its inhibitory effect, but instead functions by regulating coenzyme Q10 (CoQ10) [22]. Acetylated FSP1 is targeted to the plasma membrane and CoQ10 stimulates NAD(P)H to shuttle into the lipid bilayer with the help of FSP1; then, free radicals in the droplets are regulated to capture antioxidants, maintaining the quality of lipids and preventing LPO from inducing ferroptosis [23]. Currently, relatively few studies have examined the BH4 pathway. BH4 participates in the antioxidant system and the overexpression of its rate-limiting enzyme GTP cyclohydrolase 1 (GCH1) selectively inhibits the peroxidation of some PUFAs. Therefore, ferroptosis can be inhibited through the GCH1-BH4 pathway [24,25]. The major regulators of ferroptosis and associated mechanisms are summarized (Fig. 1).

3. Ferroptosis and Other Models of Programmed Cell Death

3.1 Ferroptosis and Necroptosis

Necroptosis is a programmed form of cell death that can regulate necrosis with passive and active proinflammatory functions [26]. Numerous studies have consistently shown that ferroptosis is invariably accompanied by necroptosis [27]. The key steps involved in necroptosis include tumor necrosis factor (TNF) signaling, deubiquitination of receptor-interacting protein (RIP) 1, phosphorylation of RIP1 and RIP3, inactivation of caspase-8, and phosphorylation of mixed lineage kinase domain-like protein (MLKL) [28]. Ferroptosis can induce mitochondrial damage, resulting in the opening of the mitochondrial permeability transition pore. Subsequently, the phosphorylation of RIP1/3 was further intensified, ultimately culminating in necroptosis [29]. These findings demonstrate that there is a crosstalk between ferroptosis and necroptosis, but the regulatory relationship between necroptosis and ferroptosis still requires further study.

3.2 Ferroptosis and Autophagy

Autophagy refers to the process where the detached double membrane of the ribosome-free attachment zone of the rough endoplasmic reticulum envelops cytoplasm, organelles, proteins, and other cellular components that require degradation to form autophagosomes [30]. Subsequently, the autophagosomes fuse with lysosomes to form autophagolysosomes, wherein the enclosed contents are degraded to fulfill the metabolic requirements and facilitate organelle renewal. A growing body of evidence suggests that ferroptosis relies on the autophagy machinery for its execution, making it a form of autophagy-dependent cell death [31]. Recent studies have discovered that autophagy plays a role in promoting ferroptosis by degrading the ferroptosis-related protein, ferritin, through the autophagy-related gene (ATG) 5/ATG7-nuclear receptor coactivator 4 (NCOA4) autophagic pathway [32]. Moreover, ras-related protein Rab-7a (RAB7A)-mediated lipophagy, BECN1-mediated system Xc^- inhibition, signal transducer and activator of transcription 3 (STAT3)-mediated lysosomal cell death, and sequestosome 1 (SQSTM1)-mediated clockophagy are involved in the regulation of ferroptosis [33]. All of these studies highlight the regulatory role of autophagy in ferroptosis, presenting a promising new treatment hypothesis by targeting autophagic ferroptosis.

3.3 Ferroptosis and Apoptosis

Apoptosis is a well-established mode of programmed cell death involving both extracellular and intracellular pathways [34]. The extracellular pathway is triggered by receptors on the cell membrane, such as TNF-α, while the intracellular pathway is primarily influenced by the permeability of the mitochondrial membrane and the regulation of the Bcl-2 protein family. Indeed, several studies have reported an interrelationship between ferroptosis and apoptosis that apoptosis can be converted to ferroptosis [35]. In addition, the ferroptosis inducer, erastin, activates the p53-dependent CHOP/PUMA pathway, enhancing sensitivity to apoptosis [36]. These findings indicate the existence of close link between ferroptosis and apoptosis.

3.4 Ferroptosis and Pyroptosis

Pyroptosis, also known as cell inflammatory necrosis, is a form of programmed cell death that relies on inflammatory caspases [37]. Pyroptosis is characterized by nuclear pyknosis, cell swelling, the formation of lipid membrane vacuoles at the plasma membrane, and eventual cell rupture without DNA fragmentation. Both pyroptosis and

ferroptosis are associated with damage to the cytoplasmic membrane. Notably, ROS-mediated damage to the cytoplasmic membrane may contribute to reciprocal regulation between pyroptosis and ferroptosis. Furthermore, research has revealed that both types of cell death can be triggered by elevated levels of intracellular iron and ROS [38]. The aforementioned study further demonstrates that a synergistic relationship and crosstalk is existed between pyroptosis and ferroptosis, but the underlying mechanisms still need to be thoroughly investigated.

4. Relationship between Ferroptosis and NAFLD

The liver, an important organ regulating lipid balance, is critical for the metabolism of lipids. Meanwhile, the liver also maintains iron homeostasis in the body and constitutes the main organ that stores excess iron and synthesizes hepcidin. In case of an imbalance between energy intake and consumption or abnormal energy storage function in adipocytes, the liver is most inclined to accumulate fat leading to hepatic steatosis, which subsequently causes systemic metabolic disorders [39,40]. In this regard, the disruption of iron metabolism is correlated with obesity and insulin resistance (IR) in patients with NAFLD. Furthermore, disturbed iron metabolism has close correlations with the typical features of obesity and pancreatic resistance in NAFLD patients [41]. The "two-hit" and "multiple-hit" hypotheses are classic theoretical explanations for NAFLD pathogenesis [42]. Major risk factors for NAFLD are considered to include IR, altered lipid metabolism, ER stress, mitochondrial dysfunction, lipid toxicity, inflammatory cascade, genetic susceptibility, epigenetic changes and disturbed intestinal flora [43]. Thus, LPO may aggravate ferroptosis. Recently, ferroptosis was suggested to play a critical role by effectively promoting and inhibiting ferroptosis-related factors in NAFLD and NASH mice [44,45]. In NAFLD, this is mainly associated with the LPO reaction triggered by iron overload and oxidative stress.

4.1 Iron Overload

Iron participates in multiple cellular processes, including oxygen storage and transport, mitochondrial respiration, DNA replication, and intercellular signaling in the body [46]. Intracellular iron balance considers iron absorption, output, use and storage. Excess iron is mostly stored in ferritin, as a redox inactive form. Generally, Fe^{3+} is bound to transferrin and transferrin receptor 1 (Tfr1) in cells and stored in the form of Fe^{3+}-ferritin complex [47]. Subsequently, hepcidin synthesized in the liver is important in maintaining iron homeostasis by controlling ferritin synthesis. A small quantity of Fe^{2+} generate a labile iron pool [48]. Iron metabolism disorders are mainly divided into two categories, including iron overload and deficiency. In case of iron overload in cells, Fe^{3+} is reduced to Fe^{2+} in lysosomes and released into the labile iron pool through the divalent metal transporter 1. Free Fe^{2+}, with high oxidative potential, easily undergoes the Fenton reaction with lipid peroxides, produces hydroxyl radicals, induces oxidative stress, enhances ROS accumulation and induces ferroptosis [49]. Fe^{2+} is thus also considered a cofactor increasing the activities of multiple metabolic enzymes, promoting lipid ROS synthesis and enhancing ferroptosis. In addition, nuclear receptor coactivator 4 (NCOA4) can mediate ferritinophagy, which dissociates the Fe^{3+}-ferritin complex to release Fe^{2+} into LIP [50,51]. Iron metabolism disorder may therefore induce the production of large amounts of Fe^{2+}, which is involved in the conversion of PUFAs through the LPO process, aggravating NAFLD, diabetes, IR and other diseases. In conclusion, iron is a crucial factor in ferroptosis, which requires iron metabolism.

Hepatocytes are critical for liver iron homeostasis. Iron shortage in hepatocytes and excessive iron accumulation in hepatic stellate cells mediated by Kupffer cells-induced secretion of iron-rich extracellular vesicles (EVs), can lead to NAFLD and NASH. Meanwhile, blocking EV secretion or EV iron transport can restore iron balance in the liver and alleviate NAFLD/NASH-associated hepatic steatosis and fibrosis. A previous study revealed that the lack of hepcidin inhibitor matriptase-2 may alleviate obesity, accelerate lipolysis, improve glucose tolerance and insulin sensitivity, and prevent hepatic steatosis in high-fat diet mice [52]. This suggests that regulation of iron homeostasis in the liver has a significant correlation with antisteatosis.

4.2 Oxidative Stress

Oxidative stress describes an imbalance between oxidative and antioxidant events, where the generation of free radicals exceeds the capacity of antioxidant defense systems. Iron overload promotes the production of ROS, thereby inducing oxidative stress. Furthermore, oxidative stress-related transcription factors downregulate or upregulate ferroptosis-related genes, which regulates ferroptosis [53–55]. The "multiple-hit" hypothesis considers oxidative stress one of the key mechanisms underlying NAFLD-related liver injury. When ROS are excessively produced through the external environment (e.g., physical and chemical factors) and the internal environment (e.g., mitochondrial dysfunction, ER stress and peroxidation), they target the double bonds of PUFAs, resulting in LPO and the formation of lipid peroxides, which induces ferroptosis and aggravates hepatocyte injury. Recent evidence suggests mitochondrial ROS play a role in NASH-related ferroptosis. Epigallocatechin gallate prevents liver lipotoxicity by suppressing mitochondrial ROS-related liver ferroptosis [41]. Quercetin reduces lipid accumulation, hepatic lipotoxicity and LPO in high-fat diet mice, ultimately inhibiting ferroptosis to alleviate NAFLD [56]. Besides, obeticholic acid reduced the amounts of triglyceride-containing PUFAs,

while elevating free PUFAs and phosphatidylethanolamine-containing PUFAs, which are prone to oxidization to generate lipid peroxides, triggering ferroptosis in hepatocytes and causing hepatic stellate cell activation [57]. Taken together, both oxidative stress and iron overload can induce the LPO process, and even synergically worsen the degree of cell damage, resulting in NAFLD progression from simple hepatic steatosis to inflammation and then fibrosis.

5. Associations of Ferroptosis with the Main Regulatory Factors of NAFLD

5.1 Glutathione

GSH is the main antioxidant in mammalian cells, which is composed of glutamate, cysteine and glycine, and represents a cofactor of GPX4 [58]. However, GSH depletion disrupts the dynamic balance of intracellular redox activity, which leads to ROS accumulation and aggravates ferroptosis [59,60]. Cysteine is the main component and thus the limiting amino acid in GSH biosynthesis; suppressing cysteine import via the cystine/glutamate antiporter SLC7A11 induces ferroptosis via GSH depletion [61]. Therefore, GSH is often used as an evaluation indicator of oxidative stress, especially in studies developing drug therapies for ferroptosis-induced NAFLD.

5.2 Glutathione Peroxidase 4

GPX4 was the first discovered selenium-containing protein inhibiting ferroptosis. It is mainly found in the cytoplasm and mitochondria, and plays a role in scavenging lipid hydrogen peroxide. GPX4 can convert toxic lipid hydroperoxides into corresponding nontoxic lipid alcohols, which forms a defense mechanism against LPO and plays an important regulatory role in ferroptosis [62]. In methionine and choline deficient L-amino acid diet (MCD)-fed NAFLD mice treated with the ferroptosis inducer (1S,3R)-RSL3, liver GPX4 was downregulated while it was upregulated by lipoxygenase and an apoptosis inducer. Subsequently, supplementation of the GPX4 activator sodium nitrite increased GPX4 expression and reduced the severity of NASH. These results indicated that GPX4 plays a significant role in ferroptosis-related changes in hepatocytes that cause NASH [63]. Therefore, GPX4 regulation may control the development of NAFLD inflammation before it progresses to NASH. NAFLD rats were treated with ferroptosis inducer (erastin) and inhibitor (ferrostatin 1 [Fer-1]), and steatosis in human liver cell 7702 (L02) was induced by palmitic acid (PA), respectively. Both in cultured cells and experimental animals, interfered GPX4 expression decreased the protective effects on rat liver and mitochondrial membrane integrity in L02 cells, and also regulated B-cell lymphoma-2 (Bcl-2), Bcl-2 associated x (Bax), Caspase-3 and superoxide dismutase 1 (SOD1), attenuating the treatment effect in NAFLD. This study revealed that suppressing GPX4-related ferroptosis may provide a novel approach for NAFLD treatment [64].

5.3 Xc^- system

The Xc^- system is known as the cystine/glutamate transporter on the cell membrane; its light chain subunit SLC7A11 is critical for the metabolism of lipid ROS. Recent data revealed p53 downregulates SLC7A11 to markedly affect the metabolism of lipid ROS in ferroptosis [65,66]. High SLC7A11 expression promoted tumor growth partially by inhibiting ferroptosis. Nevertheless, malignant cells highly expressing SLC7A11 also endure the substantial burden imposed by SLC7A11-indcued metabolic reprogramming, resulting in enhanced dependence on glucose and glutamine, which represents possible metabolic vulnerabilities for target therapies in malignant diseases [67]. Sorafenib attenuates liver damage and extracellular matrix accumulation in CCl4-dependent liver fibrosis, with concomitant SLC7A11 and GPX4 downregulation at the protein level. SLC7A11 is upregulated by hypoxia inducible factor (HIF)-1α, and HIF-1α/SLC7A11 pathway suppression is essential in sorafenib-related ferroptosis in hepatic stellate cells, ultimately limiting antifibrotic activity [68]. Furthermore, BECN1 combined with SLC7A11 can directly block the activity of the Xc^- system, indicating that SLC7A11 is a core component of the transporter [69]. As an inducer of Nrf2, SLC7A11 accelerates the transport of cystine and glutamate, promotes the synthesis of GSH, and thus achieves the purpose of suppressing ferroptosis [70,71].

5.4 Nuclear Factor Erythroid 2-Related Factor 2

Nrf2 interacts with nuclear factors to activate downstream transcription, which regulates antioxidative events and detoxification [72]. Nrf2 regulates multiple biological events, e.g., the transcription of the antioxidant system, the detoxification of endogenous and exogenous substances, NADPH regeneration and heme metabolism, as well as many cell processes, including autophagy and unfolded protein response [73]. Mounting evidence suggests a regulatory role for Nrf2 in ferroptosis. Nrf2 constitutes an upstream effector of some essential ferroptosis-related genes such as GPX4 and Xc^- system [74]. Consequently, Nrf2 negatively regulates ferroptosis and promotes ferroptosis resistance. After Kelch-like ECH-associated protein 1 (KEAP1) activates Nrf2 in oxidative stress, it recognizes antioxidant response elements and activates antioxidant genes, including SLC7A11. Furthermore, it upregulates antioxidant proteins associated with ferroptosis, e.g., heme oxygenase-1 (HO-1) and GPX4, and increases GSH levels [75,76]. A recent study reported Nrf2 activation by iron-dependent, mitochondrial pro-oxidants, which upregulates bone morphogenetic protein 6 (Bmp6) in hepatic sinusoidal endothelial cells and increases the synthesis of hepcidin in adjacent hepatocytes. In Nrf2-deficient mice, impaired Bmp6-hepcidin axis was reported, causing iron accumulation and worsened liver damage. Meanwhile, pharmacological induction of Nrf2 resulted in activated Bmp6-hepcidin signaling, ameliorating iron home-

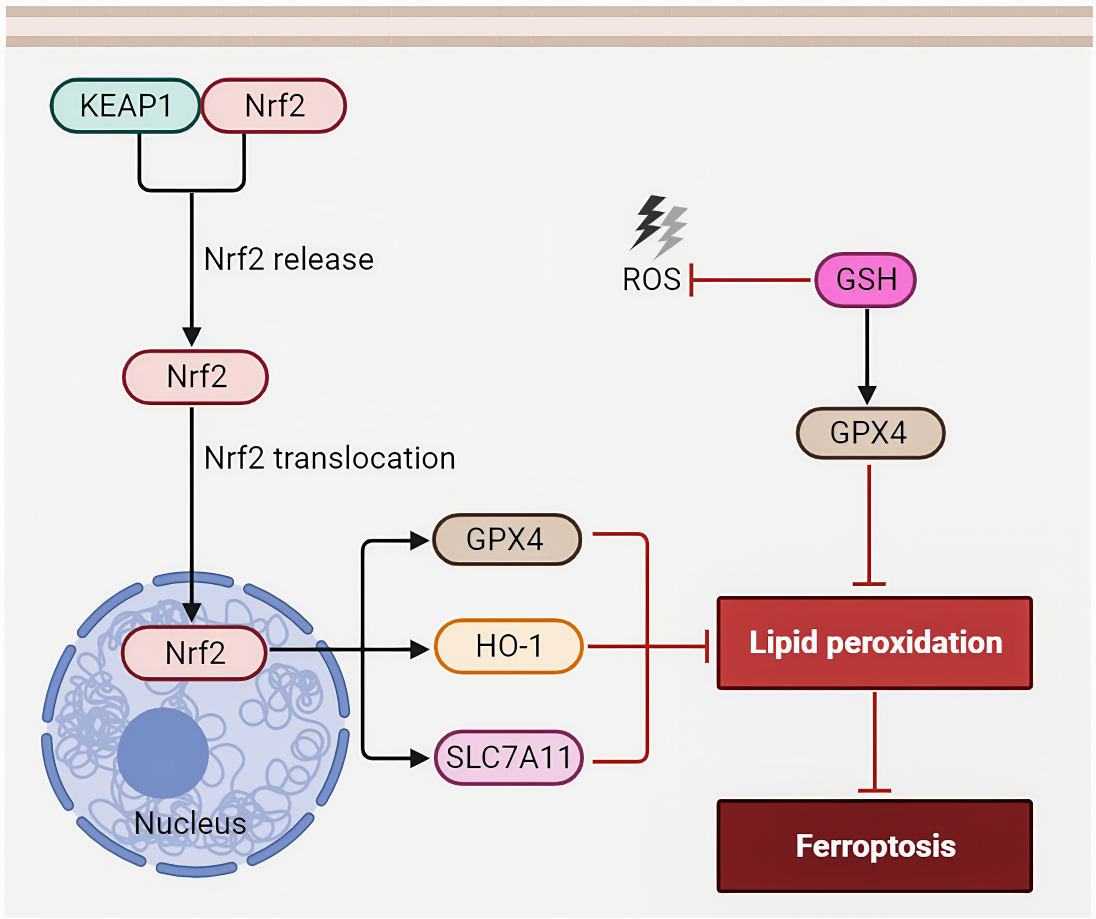

Fig. 2. KEAP1-Nrf2 pathway. When cells experience oxidative stress, the KEAP1-Nrf2 interaction is altered, preventing KEAP1 from targeting Nrf2 for degradation. As a result, Nrf2 is stabilized and accumulates in the cell. One of the key mechanisms by which Nrf2 contributes to protecting cells from ferroptosis is through the upregulation of genes involved in iron metabolism. Nrf2 induces the expression of GPX4, HO-1, and SLC7A11, releasing iron in a controlled manner. By regulating iron levels, Nrf2 helps prevent the accumulation of toxic levels of labile iron that can catalyze lipid peroxidation, a hallmark of ferroptosis. Abbreviations: KEAP1, Kelch-like ECH-associated protein 1; GPX4, glutathione peroxidase 4; HO-1, heme oxygenase-1; SLC7A11, subunit solute carrier family 7 member 11; Nrf2, nuclear factor erythroid 2-related factor 2.

ostasis and countering Bmp6 suppression [77]. Overall, Nrf2 links cellular sensitivity to iron toxicity, systemic iron homeostasis and antioxidation, representing a potential target for treating iron-related diseases.

Besides, the Nrf2 contributes to reducing ROS overproduction to exert anti-inflammatory effects for NAFLD. Indeed, a small interfering RNA (siRNA) of Nrf2 or a glutathione peroxidase suppressor could abolish nuclear factor kappa-B (NF-κB) repression and decrease the secretion of inflammatory cytokines [78]. As shown in Fig. 2, downstream effectors of Nrf2, such as HO-1, GSH and GPX4, were upregulated to reduce MDA, inhibit ROS accumulation, LPO and reduce high-fat diet-induced NAFLD [79].

5.5 Ferroptosis Suppressor Protein 1

In NAFLD, liver inflammation is a critical component of disease progression. FSP1 has been associated with the regulation of inflammatory responses in various cell types. Its involvement in immune cell migration and activation may influence the extent of liver inflammation in NAFLD [18]. FSP1 was initially identified as the homologous regulation factor of the mitochondrial pro-apoptotic protein apoptosis-inducing factor (AIF)/AIF mitochondrion-associated 1 (AIFM1), termed AIFM2. However, it was later confirmed that FSP1 lacks the N-terminal sequence for mitochondrial targeting, is not localized in mitochondria and does not promote apoptosis. It was then renamed by Bersuker *et al.* [23] as FSP1 and its cellular function was described. FSP1 is present in lipid droplets and cell membranes, and it acts as a suppressor of ferroptosis by reducing lipid peroxidation and protecting cells from oxidative damage. Mechanically, CoQ10 was identified as a major substrate using a ferroptosis activator [80]. FSP1 expression shows a positive correlation with resistance to GPX4 suppressors, and plays a critical role in maintaining cancer cell proliferation in case of no GPX4 expression. Notably, sim-

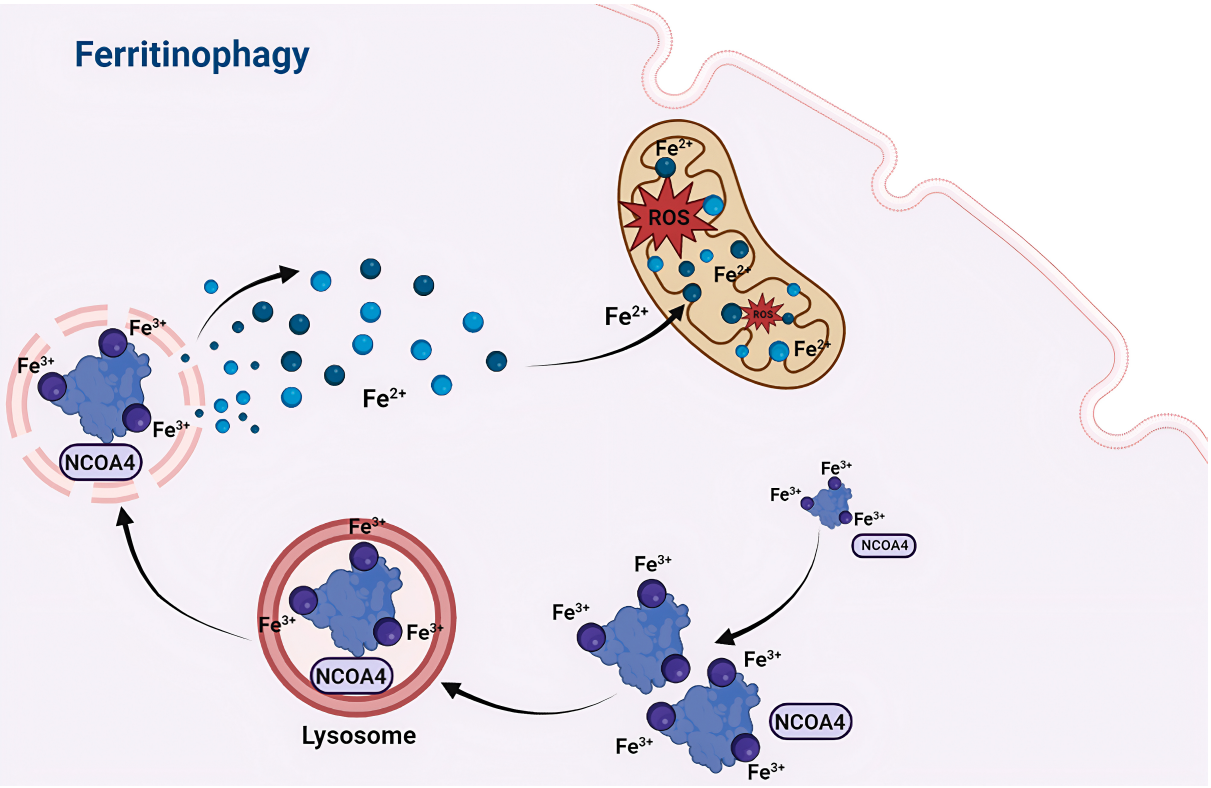

Fig. 3. Mechanism of ferritinophagy. NCOA4-mediated ferritinophagy is a selective autophagic process. NCOA4 acts as a selective cargo receptor, specifically recognizing ferritin and targeting it to autophagosomes for degradation in lysosomes. Through ferritinophagy, intracellular ferritin is broken down, releasing free iron, which can contribute to the initiation of ferroptosis. Abbreviations: NCOA4, nuclear receptor coactivator 4.

ilar to FSP1, GCH1 was recently described as a new protein suppressing ferroptosis based on whole-genome activation screening [81]. GCH1 overexpression occurs in presence of all three ferroptosis activators, including RSL3, erastin and GPX4. High GCH1 amounts enhance BH4 and CoQ10 levels, synergistically acting with the FSP1 pathway to hamper the LPO process. GCH1 expression and resistance to ferroptosis are overtly correlated, suggesting the GCH1-BH4-phospholipid pathway as a potential therapeutic target in iron-related diseases.

6. Iron Metabolism Regulatory Factors

Iron metabolism regulatory factors are essential proteins and molecules that play key roles in maintaining the balance of iron levels within cells and tissues. These factors are also known to have a close implications in NAFLD. Understanding its roles in NAFLD, such as Tfr1 and Ferritin, is crucial for elucidating the mechanisms underlying the disease and potentially identifying new therapeutic targets. They are tightly regulated by various iron metabolism regulatory factors that cells have access to adequate iron for their functions while preventing iron overload, which can lead to cellular damage and contribute to the development of iron-related NAFLD.

6.1 Transferrin Receptor 1

Transferrin receptor 1 (Tfr1), or cluster of differentiation 71 (CD71), interacts with transferrin and exerts an important effect on iron uptake by hepatocytes in a specific manner [82]. In palmitate-induced IR, palmitate simultaneously upregulates Tfr1 and induces intracellular ferroptosis. Knockdown of Tfr1 prevented palmitate-induced iron uptake and IR, and also translocated Tfr1 via enhancement of calcium influx. However, supplementation of a calcium chelator substantially decreased ferroptosis by suppressing Tfr1 translocation, and ultimately enhanced insulin sensitivity [83]. It was also found that the interaction between complexes was neutralized by antibodies against Tfr1, making Tfr1 a potential target for ferroptosis alleviation in NAFLD [84].

6.2 Ferritin

Excess iron in the body is stored in ferritin that plays a critical role in iron metabolism and storage in cells. It is usually involved in ferritinophagy which releases Fe^{3+} and is subsequently converted to Fe^{2+} to the labile iron pool, participating in the process of cell ferroptosis. Eventually, it sequesters excess iron in a non-toxic and bioavailable form to prevent the free iron from participating in harmful Fenton reactions that generate highly reactive and toxic hydroxyl

Table 1. Major inhibitors of ferroptosis in NAFLD and other diseases.

Compound	Possible mechanisms	Functions and diseases
Deferiprone	As an iron chelating agent, has affinity with Fe^{3+}	Alleviates NASH and fibrosis [45]
Ferrostatin-1	Prevents lipid ROS accumulation and inhibits LPO, constituting a lipid ROS scavenger	Alleviates NAFLD/NASH [94]
Ginkgolide B	Promotes GPX4 and FTH1 expression, and reduces TFR1 expression, ROS levels, and intracellular iron content	Improves fibrosis and diabetic nephropathy [95]
Trolox	Damps lipoxygenase and abrogates hydroxyl group radicals, with potent antioxidant properties	Revealed an association between blood redox status and the likelihood of NAFLD [96]
Liproxstatin-1	Functions as a radical-trapping antioxidant to inhibit lipid peroxidation	Ameliorates NAFLD and acute renal failure [45,97,98]
Selenium	Increases the expression of GPX4	Affects the progression of NASH [99]
CoQ10	Acts as a lipophilic radical-trapping antioxidant to prevent the generation of lipid peroxides	Exerts hepatoprotective effects and is beneficial in NAFLD; sensitizes malignant cells to ferroptosis-activating chemotherapeutics [23,100]
Baicalein	Targets lipoxygenases and suppresses lipoxygenase-triggered LPO	Exerts pharmacological effects in bladder cancer, vitiligo and ischemia-reperfusion injury [101–103]
XJB-5-131	Exerts antioxidant effects and suppresses LPO	Improves ischemia-reperfusion injury and acute kidney injury [104,105]

NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; LPO, lipid peroxidation; CoQ10, coenzyme Q10; GPX4, glutathione peroxidase 4; FTH1, ferritin heavy chain 1.

radicals [85]. Therefore, suppressing ferritinophagy may provide a novel approach and target for treating NAFLD [86]. Elevated Ferritin levels are commonly observed in patients with NAFLD. It is important to note that the relationship between Ferritin and NAFLD is complex and multifactorial, involving iron metabolism, oxidative stress, and inflammation. It was reported that prominin-2, a pentapeptide protein associated with lipid homeostasis, is induced by ferroptosis response and induces the generation of ferritin-containing multivesicular bodies (MVBs), which transports iron out of cells to suppress ferroptosis. This suggests the prominin-2/MVBs-ferritin pathway has certain significance in iron homeostasis and intracellular transport [87]. Therefore, further investigation is needed to study how to inhibit ferroptosis-induced hepatic steatosis from the perspective of iron metabolism.

7. Ferritinophagy-Mediated Ferroptosis

Recently, ferroptosis has attracted increasing attention among investigators studying liver pathologies and was shown to involve the regulation of NCOA4-mediated ferritinophagy [88]. There is a feedback mechanism behind intracellular iron utilization in which NCOA4-mediated ferritinophagy degrades ferritin, and its activation increases the amounts of available iron within the cell (Fig. 3). In terms of mechanism, the selective autophagy receptor NCOA4 interacts with ferritin heavy chain 1 (FTH1) and mediates ferritin transfer to autolysosomes, eventually releasing free iron. Such regulatory effect is not unidirectional, as intracellular iron levels also affect the flux of ferritinophagy [89]. In case of high iron content, HECT and RLD domain containing E3 ubiquitin protein ligase 2 (HERC2), an enzyme 3 (E3) ubiquitin ligase, drives ubiquitin-dependent degradation of NCOA4. This process reduces the ferritinophagy flux, thereby maintaining iron homeostasis [90]. It is noteworthy that NCOA4 level is a central determinant of the ferritinophagy flux. In the non-disease state, ferritinophagy maintains cellular iron balance, but its overactivation can cause iron overload within cells. LPO, which is correlated with iron overload, is an important factor required for ferroptosis [89]. The above findings jointly suggest a close relation between ferritinophagy and ferroptosis. A study confirmed GPX4 suppresses ferroptosis and shows elevated expression in NCOA4-knockout mice, revealing an association of ferroptosis with ferritinophagy [91]. Additionally, several studies have suggested that ferritinophagy promotes ferroptosis, primarily because of iron overload resulting from NCOA4 upregulation. Sorafenib is an iron-dependent inducer of ferroptosis, whose potential mechanism involves increasing NCOA4 expression to accelerate iron overload and ROS generation [92]. Moreover, elevated iron content induced by NCOA4 overexpression increases sensitivity to ferroptosis. Conversely, ferritinophagy protects from ferroptosis. In case of NCOA4 deficiency or obstructed NCOA4 binding to FTH1, ferritinophagy is hindered, which may decrease sensitivity to ferroptosis within cells [32]. Furthermore, NCOA4 knockout blocks sideroflexin 1-mediated mitochondrial iron overload, thereby inhibiting ferroptosis

Table 2. Major inducers of ferroptosis in NAFLD and other diseases.

Compound	Possible mechanisms	Functions and diseases
RSL-3	Suppresses GPX4 and induces the accumulation of lipid hydroperoxides	Aggravates gastric cancer, hepatocellular carcinoma, triple-negative breast cancer, and colorectal cancer [106–109]
High-fat diet	Promotes iron overload and oxidative stress, and inhibits the expression of GPX4 and SLC7A11	Aggravates NASH [110]
Erastin	Targets the Xc^- system and decreases GSH	Weakens therapeutic effects in NAFLD and enhances the growth of tumors, e.g., melanoma and triple negative breast cancer [111,112]
Sorafenib	Suppresses cystine uptake through the Xc^- system and decreases GSH	Ameliorates hepatocellular carcinoma and murine liver fibrosis [67,113]
Sulfasalazine	Suppresses cystine uptake through the Xc^- system and decreases GSH	Improves mesenchymal traits and disabled antioxidant program in head and neck cancer, hepatocellular carcinoma and pancreatic cancer [114,115]
Glutamate	High concentrations of glutamate inhibit cystine uptake by the Xc^- system	Improves non-small cell lung cancer [116]
ML-162	Suppresses GPX4 and causes disorders of lipid hydroperoxides	Improves head and neck cancer [117]
FIN 56	Decreases GPX4 expression, activates squalene synthase and suppresses CoQ10	Exerts anticancer effects in human fibrosarcoma and head and neck cancer [117,118]
Dihydro-artemisinin	Decreases GPX4 expression	Increases sensitivity to ferroptosis in cancer cells [119]
Ferric ammonium citrate	Causes iron overload and LPO	Protects against iron overload-associated knee osteoarthritis and osteoblast differentiation [120,121]
Trigonelline	Inhibits Nrf2	Reduces blood glucose and kidney injury in diabetes, and renders cancer cells more susceptible to apoptosis [122,123]
FINO(2)	Suppresses GPX4-related enzymatic activity and oxidizes iron, and causes LPO	Exerts anticancer effects in human fibrosarcoma [124]
Brequinar	Inhibits DHODH and thus decreases ubiquinone reduction to ubiquinol	Suppresses renal, cervical and nasopharyngeal cancer growth [125,126]

RSL-3, (1S,3R)-RSL3; SLC7A11, subunit solute carrier family 7 member 11; DHODH, dihydroorotate dehydrogenase.

[93]. Taken together, these data indicate iron overload represents the initial step of ferritinophagy-mediated ferroptosis. The major factor in ferritinophagy regulation is to influence its core molecule NCOA4 by controlling its expression or preventing its interaction with ferritin.

Overall, NCOA4-dependent ferritinophagy exerts a crucial regulatory effect on ferroptosis by modulating iron homeostasis in cells and controlling ROS biosynthesis. Further research is necessary to investigate the pathways by which ferritinophagy modulates ferroptosis and to develop drugs that may target ferritinophagy as a therapeutic strategy to ameliorate the pathological processes associated with ferroptosis in various diseases.

8. Pharmacologic Regulation of Ferroptosis and Ferritinophagy in NAFLD and Other Diseases

Agents selectively regulating ferroptosis (inhibitors or inducers) are crucial for the mechanistic studies of ferroptosis-dependent NAFLD and other disorders. Table 1 (Ref. [23,45,94–105]) and Table 2 (Ref. [67,106–126]) list the best-studied and most widely-applied inhibitors and inducers of ferroptosis, respectively.

Ferroptosis plays a crucial role in liver pathophysiology and is also a key factor in initiating the progression from NAFLD to NASH. Therefore, it holds great promise as a potential therapeutic target for preventing the development of NAFLD. While the existing data strongly suggest that targeted ferroptosis could be an excellent approach for treating NAFLD, it is essential to acknowledge the current gaps in research on this topic. To facilitate its understanding and potential applications, certain issues should be addressed in the future. These mainly include: (1) elucidating the mechanisms: further researches are needed to elucidate deeper and detailed molecular mechanism that are aiming to pave the way for more effective and targeted therapeutic interventions; (2) identifying biomarkers: in the context of NAFLD, the identification of reliable biomarkers asso-

ciated with ferroptosis in NAFLD is essential for early detection and diagnosis; (3) addressing specific subtypes: investigating how ferroptosis contributes to the progression of different NAFLD subtypes will aid in tailoring treatments to specific patient populations; (4) investigating combination therapies: considering wide spectrum in NAFLD, exploring the combinations involving ferroptosis-targeting agents along with other existing treatments may offer a more comprehensive approach to management. By addressing the aforementioned gaps, we may have the opportunity to fully unleash the potential of targeted ferroptosis as a powerful strategy for effectively preventing and treating NAFLD.

9. Conclusions

Ferroptosis represents a newly discovered form of cell death associated with iron-dependent LPO. Currently, studies exploring ferroptosis mechanistically in human health are mainly focused on cancer, and based on reported mechanisms, new targets for developing anti-cancer drugs have been discovered. Summarizing recent studies on the pathways and defense mechanisms associated with ferroptosis, accumulating evidence indicates a certain correlation among the pathological processes of NAFLD such as lipid accumulation, hepatic steatosis, inflammatory cell infiltration, fibrosis, mitochondrial damage and ferroptosis. Iron overload and oxidative stress-induced LPO may mediate the progression of NAFLD, and the main antioxidant mechanism and regulatory factors controlling iron metabolism may become potential targets for NAFLD-related research. Among them, targeting Nrf2 activation can cause SLC7A11 to accelerate the transport of cysteine and glutamate through the Xc^- system, promoting GSH synthesis. GSH, the most important endogenous antioxidant in the human body, is involved in the downregulation of GPX4, which suppresses LPO and prevents ferroptosis.

With multiple potential targets and overarching approaches for regulating ferroptosis emerging from recent reports, ferritinophagy intervention could pave a novel way for ferroptosis regulation. Ferritinophagy-mediated ferroptosis is a potential target for preventing the onset of NAFLD. Therefore, it is necessary to also take ferritinophagy into account in strategies for NAFLD therapy. The concept of ferroptosis has enormous research potential and clinical value in the field of NAFLD. This review provides new insights into ferroptosis relevant to the pathogenesis of NAFLD. However, more detailed mechanism of action for ferroptosis in NAFLD still needs further investigation.

Abbreviations

4-HNE, 4-hydroxynonenal; ACSL4, acyl-CoA synthetase long chain family member 4; Bmp6, bone morphogenetic protein 6; CD71, cluster of differentiation 71; CoQ10, coenzyme Q10; ECM, extracellular matrix; ER, endoplasmic reticulum; ETC, electron transport chain; EVs, extracellular vesicles; Fer-1, ferrostatin 1; FSP1, ferroptosis suppressor protein 1; FTH1, ferritin heavy chain 1; GCH1, GTP cyclohydrolase 1; HO-1, heme oxygenase-1; IR, insulin resistance; GSH, glutathione; KEAP1, Kelch-like ECH-associated protein 1; LPCAT3, lysophosphatidylcholine acyltransferase 3; LPO, lipid peroxidation; MDA, malondialdehyde; MVBs, multivesicular bodies; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NCOA4, nuclear receptor coactivator 4; Nrf2, nuclear factor erythroid 2-related factor 2; PA, palmitic acid; PL·, phospholipid radical; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; TCA; tricarboxylic acid; Tfr1, transferrin receptor 1.

Author Contributions

SNZ and XZY designed the study. SNZ, YG and XZY collected and analyzed the literatures. SNZ and XZY wrote the manuscript. YG and XZY discussed and critically revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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