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Advancements in ferroptosis research and therapeutic strategies for alcoholic liver disease: a narrative review

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Abstract. – Ferroptosis is a novel mechanism of programmed cell death characterized by an iron overload-induced lipid peroxidation cascade. The incidence of alcoholic liver disease (ALD) is rising globally, contributing to markedly high morbidity and mortality. ALD pathogenesis is an intricate and continuously evolving process. Several basic and clinical investigations have established a correlation between ferroptosis and ALD initiation and progression. Additionally, anti-ferroptosis drugs have demonstrated effectiveness in ameliorating alcohol-induced liver injury. This review aims to provide an overview of recent advancements in ferroptosis research pertaining to ALD, encompassing imbalance of antioxidant systems, iron overload, autophagy, mitochondria, epigenetic changes, and prospective therapeutic drugs targeting ferroptosis. Our aim is to reveal the potential of ferroptosis-related diagnoses and therapeutic interventions for the treatment of ALD.

Key Words:
Ferroptosis, Alcoholic liver disease, Lipid peroxidation, Iron overload, Autophagy.

Abbreviations

TLR4, toll-like receptor 4; BMP6, bone morphogenetic protein 6; SMAD4, SMAD family member 4; Nrf2, Nuclear factor erythroid2-related factor 2; NF-κB, nuclear factor kappa-B; PUFAs, Polyunsaturated fatty acids; DMF, Dimethyl fumarate; PI3K, Phosphatidylinositol-3-kinase; Akt/PKB, protein kinase B; Keap1, Kelch-like ECH- associated protein l; PFP-1, a polysaccharide isolated from the fruiting body of Pleurotus geesteranus; CQ, chloroquine; 3-MA, 3-Methyladenine; FTH, Ferritin Heavy Chain; FPN, ferroportin, HO-1, Heme Oxygenase-1; p62, prostacyclin; SLC7A11, Solute Carrier Family 7 Member 11; DMT1, divalentmetal-iontransporter-1; ARE, anti-oxidant response elements; BMAL1, Basic Helix-Loop-Helix ARNT Like 1; NCOA4, Nuclear receptor coactivator 4; PINK1, PTEN Induced Kinase 1.

Introduction

Ferroptosis is an iron-dependent, non-apoptotic form of cell death characterized by massive accumulation of lipid peroxides. It was first proposed by Dixon et al[1] in 2012. Cells undergoing ferroptosis typically exhibit decreased mitochondrial volume, reduced or diminished mitochondrial cristae number, and condensed mitochondrial membranes; however, they maintain a normal nuclear structure[2]. Recent studies[3-5] have identified three key mechanisms underlying ferroptosis: iron metabolism disorder, antioxidant system depletion, and lipid peroxidation, all of which can serve as diagnostic evidence of ferroptosis.

Alcoholic liver disease (ALD) is a leading cause of chronic liver disease globally, which can result in fibrosis, cirrhosis, and, ultimately, hepatocellular carcinoma. In 2010, approximately 500,000 patients worldwide died from alcoholic cirrhosis, accounting for 47.9% of all cirrhosis-related deaths, with an additional 80,000 deaths attributed to alcohol-related hepatocellular carcinoma[6]. The "multiple hits" hypothesis has gained widespread acceptance in recent years as an explanation for the formation and progression of ALD, positing that multiple factors co-induce the disease[7].

Studies[8-10] in recent years have indicated that ferroptosis plays a role in the development and progression of ALD. However, the precise mechanisms through which ferroptosis contributes to ALD remain unclear, and research on ferroptosis-targeted therapies for the treatment of ALD remains limited. Therefore, a comprehensive understanding of the pivotal role of ferroptosis in ALD will provide a theoretical basis for the devel-

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opment of novel treatment strategies for ALD targeting ferroptosis. This article reviews the mechanisms underlying ferroptosis, the pathogenesis of ALD, and the involvement of ferroptosis in the pathophysiology of ALD.

Methods

Relevant research articles and reviews until March 2023 were extensively collected using the terms: "ferroptosis", "alcoholic liver disease (ALD)", "alcoholic fatty liver (AFL)", "alcoholic steatohepatitis (ASH)" from PubMed and Web of Science online database.

Mechanisms of Ferroptosis

Iron Metabolism and Overload

Iron metabolism and regulation

Iron is an essential trace element in the body, but excess free iron causes cellular damage and promotes oxidative stress and is one of the crucial elements in ferroptosis. In the body, redox-active "free" iron includes circulating non-transferrin-bound iron (NTBI)[11] and the cytoplasmic labile iron pool Fe (LIP-Fe)[12]. LIP-Fe is present in various cellular compartments, including the cytoplasm, mitochondria, and lysosome[13]. LIP-Fe can be stored in ferritin (Ft) and released extracellularly by ferroportin 1 (FPN1)[14]. Ft is directed to lysosomes for degradation by nuclear receptor coactivator 4 (NCOA4)[15]. Transient receptor potential mucolipin 1 is involved in the release of iron in late endosomes and iron complexes in lysosomes, particularly Ft, and is currently considered the primary source of cellular LIP-Fe[16]. Subsequently, LIP-Fe in the cytoplasm can be rapidly absorbed into the mitochondria by the mitochondrial calcium uniporter in the inner mitochondrial membrane[17].

Iron overload

Iron overload, resulting from an imbalance in iron input, storage, and export, has been found[18] to affect susceptibility to cellular ferroptosis. Iron and iron derivatives, such as heme or Fe-S clusters, are essential active sites for enzymes involved in reactive oxygen species (ROS) production, including lipoxygenase (LOX), cytochrome P450, and NADPH oxidase. During ferroptosis, the Fenton reaction is initiated by the interaction between Fe2+ and hydrogen peroxide to generate hydroxide and hydroxyl radicals[19]. Diverse methods of increasing free iron accumulation in cells can elevate ROS production *via* the Fenton reaction, resulting in lipid peroxidation and ultimately triggering ferroptosis[20].

Antioxidant System

Ferroptosis is counteracted by antioxidant signaling mechanisms, which mainly include the nicotinamide adenine dinucleotide phosphate (NAD(P)H-) glutathione (GSH)-glutathione peroxidase 4 (Gpx4) system, NAD(P)H-ferroptosis suppressor protein 1 (FSP1)-ubiquinone 10 (CoQ10) pathway, and dihydroorotate dehydrogenase (DHODH)-CoQ-CoQH2 pathway. Gpx4 is a selenocysteine-containing GSH-dependent enzyme. GSH is a crucial substrate for Gpx4, and plays a vital role in ferroptosis prevention. Gpx4 converts lipid hydroperoxides (R-OOH) to fatty alcohols (R-OH) by utilizing GSH as a cofactor[21], and this mechanism inhibits the generation and accumulation of toxic lipid products peroxidation. *FSP1* is an anti-ferroptosis gene that inhibits lipid peroxidation and catalyzes the regeneration of non-mitochondrial CoQ10 *via* NAD(P)H to prevent ferroptosis. CoQ10 acts as a reversible redox carrier in plasma and Golgi membrane electron transport and is an essential endogenous lipid-soluble antioxidant[22,23]. Moreover, the newly discovered DHODH is a mitochondrial inner membrane enzyme that has a parallel effect with mitochondrial Gpx4 and jointly antagonizes lipid peroxidation in mitochondria[24].

Lipid Peroxidation

Lipid peroxidation mainly refers to the peroxidation of polyunsaturated fatty acids (PUFAs) and is a fundamental process in ferroptosis. PUFAs can be incorporated into the membrane structures with polyunsaturated phospholipids in cells by acyl-CoA synthetase long-chain family member 4 (ACSL4)[25] and lysophosphatidylcholine acyltransferase 3 (LPCAT3)[26] to form PUFA-phospholipid ethanolamine (PUFA-PE). PUFA-PE can be oxidized by lipoxygenase (LOX) or through a non-enzymatic pathway to form PE-PUFA-OOH[27-29]. Lipid peroxide can be broken down into toxic derivatives such as 4-hydroxynonenals (4-HNEs) and malondialdehyde (MDA), which can cause severe cellular toxicity by reacting with DNA bases, proteins, and other nucleophiles. Moreover, once lipid peroxide is produced, it may enhance ROS signaling, exacerbate lipid peroxidation, and trigger ferroptosis[30] (Figure 1).

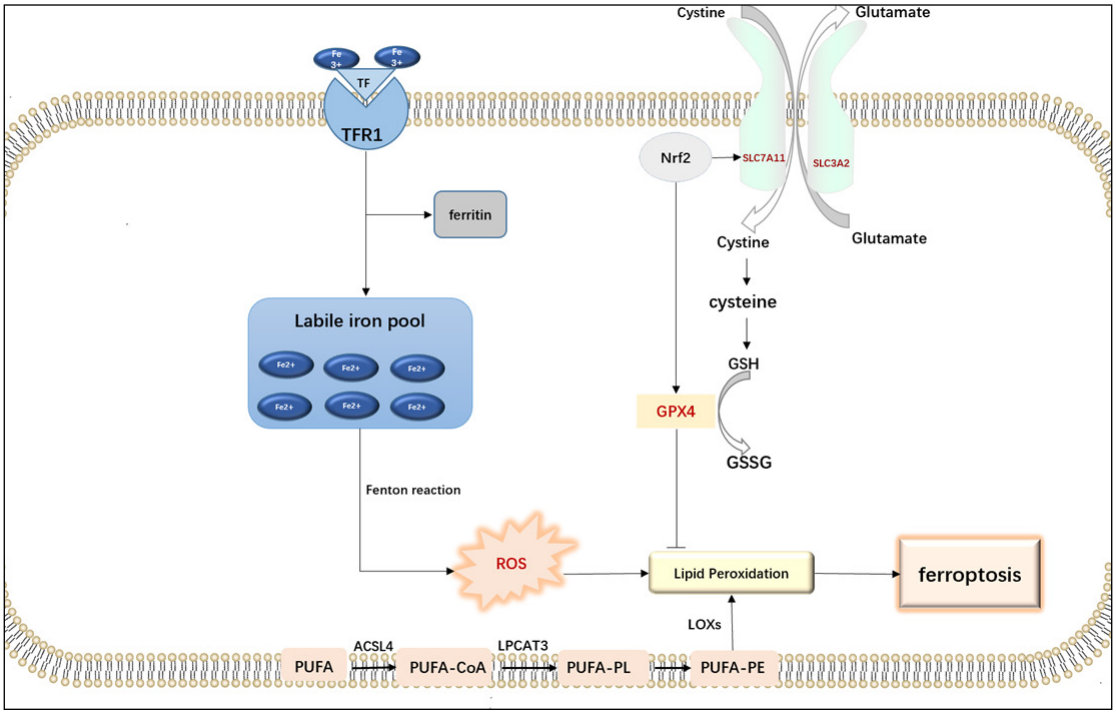

Figure 1. Mechanisms of ferroptosis.

Pathogenesis of ALD

Direct Toxicity of Acetaldehyde

Acetaldehyde, a toxic and carcinogenic by-product of alcohol metabolism, causes structural and functional changes by binding to proteins and inducing the formation of new antigens. Chronic alcohol consumption activates the microsomal ethanol oxidation system (MEOS), leading to excessive acetaldehyde synthesis[31]. Acetaldehyde-induced mitochondrial structural abnormalities result in reduced ATP production by the respiratory chain, increased ROS generation, and decreased aldehyde dehydrogenase activity, further aggravating oxidative stress[7].

Oxidative Stress

Chronic alcohol consumption primarily promotes ROS production *via* MEOS activation and alcohol-induced inflammation. ROS can cause excessive cell regeneration, lipid peroxidation, and generation of novel antigenicity by altering the functional and structural properties of proteins. The continuous expression of activating protein 1 (AP-1) transcription factor and activation of c-Jun N-terminal kinase also promote lipid peroxidation, generating products such as MDA and 4-HNE in ALD. Together with adenosine and cytosine, lipid peroxide can form highly carcinogenic exocyclic ethenoDNA adducts[32].

Antioxidant System Imbalance

Chronic alcohol consumption mediates the consumption of GSH and results in a depletion of the antioxidant system activity. The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which regulates the production of a key cytoprotective enzyme, is upregulated following chronic alcohol consumption as an adaptive response to CYP2E1-induced oxidative injury[33]. Nrf2 is a key regulator of cellular antioxidant responses and expression of antioxidant and electrophilic stress genes. Upon exposure to oxidative stress, Nrf2 activates various antioxidants, including heme oxygenase-1 (HO-1), NADPH quinone dehydrogenase 1, glutamate-cysteine ligase (GCLM/GCLC), and the glutathione peroxidase (GPX) family proteins[34,35].

Epigenetic Changes in ALD

Chronic alcohol consumption can lead to hepatic epigenetic alterations, such as DNA hypomethylation, DNA acetylation, protein phosphorylation, and alterations in microRNAs (miRNAs)[36].

Chronic alcohol intake controls histone H3 acetylation by increasing histone acetyltransferase activity and inhibiting histone deacetylase (HDAC), potentially promoting alcohol dehydrogenase isoenzyme expression[37]. In alcohol-exposed hepatocytes, the expression of sirtuin 1 (SIRT1), which is a III-HDAC NAD-dependent protein deacetylase, is decreased; it mediates hepatic steatosis and inflammatory damage. Epigenomic hypomethylation can lead to transcriptional activation, which may alter cellular functions[38]. miRNAs are also involved in the pathogenesis of ALD, and the elevated production of certain miRNAs in ALD may be associated with liver lipid metabolism, inflammatory responses, and other activities[39,40].

Additional Factors

In addition to the previously mentioned factors, chronic alcohol consumption also disrupts the mitochondrial β-oxidation of fatty acids. This is achieved by upregulating the sterol regulatory element binding protein 1c (SREBP1c), which stimulates the expression of lipogenesis genes, reducing the activity of peroxisome proliferator-activated receptors (PPARα), and inhibiting autophagy[41]. Furthermore, gut-derived pathogen-associated molecular patterns, inhibition of the ubiquitin-proteasome pathway, apoptosis, and regeneration disorders may contribute to the onset and progression of liver inflammation[42].

Ferroptosis and ALD

Ferroptosis and Antioxidant System Imbalance in ALD

GSH depletion

GSH depletion is a major contributor to hepatocyte ferroptosis in ALD. GSH, a key antioxidant amino acid, is often used as a hepatoprotective drug. The mitochondrial pool of GSH is derived from the cytoplasm. During chronic alcohol intake, the levels of mitochondrial GSH were reduced, due to the accumulation of unesterified cholesterol in the inner mitochondrial membrane, thereby increasing microviscosity and disrupting GSH transport between the mitochondria and cytosol[43]. Moreover, chronic alcohol consumption inhibits the methionine cycle and transsulfuration pathway, leading to decreased GSH synthesis and hepatic S-adenosylmethionine (SAM) utilization[44]. Notably, SAM is an intermediate product in the methionine cycle, that not only serves as a precursor for GSH but also supports mitochondrial GSH transport, protects mitochondrial integrity in the liver cells of rats with ALD, and restores the GSH mitochondrial pool[45,46].

Nrf2

Dysregulation of antioxidant genes is induced by acetaldehyde and leads to reduced production of antioxidant and detoxification enzymes[47]. Activation of the Nrf2-Kelch-1ike ECH-associated protein l (Keap1) signaling pathway can inhibit ferroptosis by upregulating system Xc-[48]. System Xc- is a Cys2/glutamate (Glu) antiporter composed of a 4F2 heavy chain (4F2hc/CD98/SLC3A2) and light chain (XCT/SLC7A11), and it has been demonstrated[49] that the inhibition of system Xc- results in reduced GSH levels and the initiation of ferroptosis. Nrf2 can regulate the glutamic acid-cysteine ligase expression by controlling AP-1 and the nuclear transcription factor-κB (NF-κB) signaling pathway, thereby stimulating GSH synthesis[50].

Ferroptosis and Iron Overload in ALD

ALD is typically associated with hepatic iron overload. In the early stages of ALD, there is a putative pathway for iron dyshomeostasis involving increased iron absorption by hepatocytes and the intestine[51]. Ethanol stimulation induces a considerable increase in hepatic iron, LIP-Fe, and serum NTBI levels[16], whereas LIP-Fe overload in hepatocytes initiates the production of excess free radicals by participating in the Fenton/Haber-Weiss reaction cycle[52].

Alcohol-induced hepatic iron overload is attributed to multiple mechanisms, including direct activation of iron-regulating proteins[53], and indirectly inhibits hepcidin transcription and expression in hepatocytes *via* numerous signaling pathways[54-57]. Depending on FPN, hepcidin downregulates the absorption of dietary iron in the duodenum and inhibits the release of iron from macrophages and hepatocytes[57,58]. Specifically, intracellular Ft degradation *via* the NCOA4-mediated autophagic pathway (ferritinophagy) liberates Ft-bound ferric ions as free ferric ions, which increases intracellular iron overload and labile iron levels[59] (Figure 2).

Nrf2 plays an important role in iron metabolism and is closely related to oxidative stress and ferroptosis. Nrf2 upregulates the expression of Ft and FPN1, thus reducing the labile iron pool[35]. Moreover, Nrf2-deficient mice show[60] reduced hepatic FPN1 and hepcidin and significantly increased ROS and MDA levels, resulting in lipid

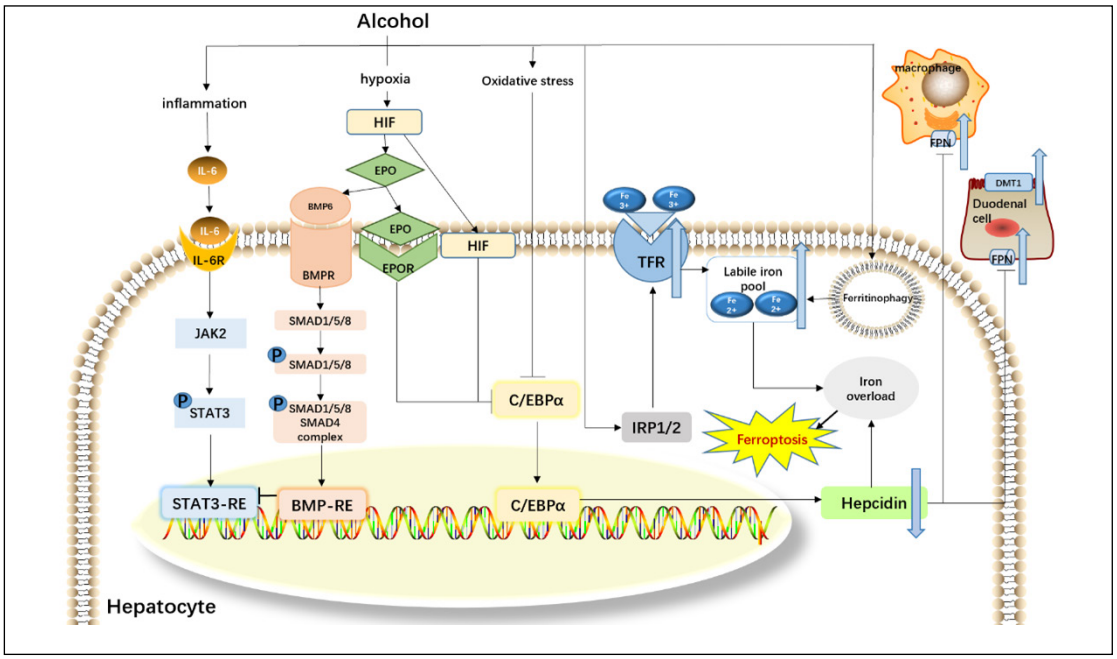

Figure 2. Ferroptosis and the molecular mechanisms of ethanol-induced iron overload in the liver.

peroxidation. Sestrin2 (SESN2), a conserved antioxidant protein, is upregulated through the Nrf2-ARE signaling pathway under oxidative stress stimulation[61]. Upregulated SESN2 can prevent iron overload, attenuate oxidative stress, and alleviate liver damage caused by ferroptosis[62].

Fibronectin type III domain-containing protein 3B (FNDC3B) is a member of the fibronectin type III domain-containing protein family and participates in energy sensing and, homeostasis, adipogenesis. FNDC3B inhibition results in Amp-activated protein kinase (AMPK) deactivation, which is linked to the inhibition of transferrin expression, resulting in ferroptosis in ALD[8].

Ferroptosis and Autophagy in ALD

Recent research[63] has established a correlation between ferroptosis and autophagy. NCOA4-dependent ferritinophagy has been demonstrated to be involved in ferroptosis in ethanol- or acetaldehyde-treated liver cell lines. In addition, Song et al[64] discovered that PTEN-induced kinase 1 (PINK1)-Parkin mitophagy can protect against alcohol-induced liver injury by inhibiting ferroptosis[65]. Notably, Zhao et al[66] found that melatonin reduces ferroptosis *via* the circadian protein ARNT-like 1 in ALD mouse models. This finding suggested that ferroptosis in ALD may be linked to Sequestosome 1-dependent clockophagy. The crosstalk between ferroptosis, autophagy, and ALD mentioned above suggests that ethanol or acetaldehyde may have opposing effects on different types of autophagy, contributing to diverse effects on ferroptosis in ALD.

Acute and chronic alcohol consumption have a distinct influence on autophagy. Autophagy protects hepatocytes and Kupffer cells against ethanol-induced liver injury by eliminating unfolded proteins and limiting lipid accumulation through adipophagy during acute ethanol exposure. The activation of Cannabinoid receptor 2 receptors suppresses liver inflammation *via* a macrophage autophagy-dependent mechanism, thereby preventing alcohol-induced hepatic steatosis[67]. However, long-term alcohol consumption inhibits autophagy through several mechanisms, including reduced mTOR activation, elevated lysosomal pH[68], impaired lysosomal enzyme trafficking[69], and decreased expression of the transcription factor EB[70], which is required for lysosomal biogenesis and autophagy.

The complex mechanism by which autophagy regulates ferroptosis in ALD requires further investigation, particularly the study of autophagy type and duration of alcohol intake. Such investigations are crucial for developing future diagnoses and treatments targeting autophagy *via* ferroptosis in ALD.

Ferroptosis and Mitochondrion in ALD

Frataxin is a mitochondrial protein that plays a crucial role in Fe-S cluster synthesis and antioxi-

dant defenses[71,72]. Related studies[73-75] have shown that frataxin deficiency lowers the threshold for triggering ferroptosis in Friedreich's ataxia. Similar conclusions were obtained in the study of Liu et al[76] on liver. They discovered that alcohol could inhibit frataxin expression *via* the upregulation of hepatic CYP2E1 activity, leading to the accumulation of ROS and mitochondrial active iron pools, resulting in ferroptosis.

CDGSH iron sulfur domain 1 (CISD1) is a 13 kDa iron-containing mitochondrial outer membrane protein[77] that regulates mitochondrial respiration by inhibiting mitochondrial Fe2+ uptake and transport to the mitochondrial matrix[78-81]. The genetic suppression of *CISD1* increases iron-mediated intramitochondrial lipid peroxidation, contributing to ferroptosis in HepG2 and Hep3B cells[82]. However, Hu et al[83] discovered that ethanol-fed *CISD1*KO mice displayed significantly lower liver damage than that displayed by ethanol-fed wild-type mice. Further investigation revealed that CISD1 deficiency increases adiponectin and fibroblast growth factor (FGF) 15 expression. The adiponectin and FGF15 upregulation contributes to the normalization of liver and serum bile acid levels, impedes the accumulation of toxic bile in the liver, activates liver SIRT1, reduces the activity of NF-κB and the expression of lipid-intake CD36, and mitigates liver inflammation. Interestingly, the increase in adiponectin and FGF15 levels and ferroptosis caused by CISD1 inhibition have opposite functions in ALD, the reasons for these conflicting effects require further exploration.

Ferroptosis and Epigenetic Changes in ALD

SIRT1, a mammalian NAD-dependent protein deacetylase, plays a critical role in the development of colitis and intestinal inflammation[84,85] and is involved in the prevention of ASH[86,87]. Ethanol-induced inhibition of hepatic SIRT1 expression mediates hepatic steatosis and inflammatory injury by disrupting a signaling network composed of multiple transcriptional regulators and co-regulators, including mTOR complex 1, sterol regulatory element binding protein-1c, PPARα, lipin-1, AMPK, adiponectin, NF-κB, and peroxisome proliferator-activated receptor gamma coactivator 1 α[86,88-90]. Adipose-specific overexpression of lipin-1 severely hinders hepatic SIRT1 expression, resulting in iron overload, decreased GSH and GPX4 levels, and elevated MDA concentrations, culminating in liver ferroptosis[10]. Similarly, C3 triggers ethanol-induced hepatic steatosis and inflammation by downregulating SIRT1 expression. In a clinical study, Zhong et al[91] found that patients with alcoholic fatty liver disease (AFLD) have lower levels of SIRT1 expression but higher hepatic C3d, glycine tRNA-derived fragment (Gly-tRF), and CYP2E1 expression than those of healthy controls. Animal experiments[91] have shown that the C3 activation product C3a or Asp (C3a-des-arg) regulates the expression of Gly-tRF through CYP2E1, and downregulates the expression of SIRT1, thereby promoting downstream lipogenesis and inhibiting the β-oxidation pathway. Conversely, studies[92] of the intestine of the ALD mouse model have shown that intestinal SIRT1 deficiency leads to elevated GSH and GPX4 levels, reduced MDA levels, partial correction of iron metabolism disturbances, and protective effects against ALD by attenuating hepatic ferroptosis.

Currently, known absorption pathways for cysteine include systemic Xc-internalization of cystine (Cys2)[93] and the transsulfuration pathway[94,95], which links GSH biosynthesis and methylation. Notably, SAM is not only a crucial intermediary in the transsulfuration route but also an essential methyl donor for DNA methylation modification, which may play a crucial role in initiating ferroptosis. Chronic alcohol intake led to epigenome hypomethylation, with one of the primary reasons being the reduction in SAM levels[38]. The possible mechanisms include alcohol-induced folate deficiency that reduces the folate cycle[96], acetaldehyde inhibition of DNA methyltransferase activity[97], and ROS formation during alcohol metabolism, which largely consumes molecules required for remethylation, resulting in decreased methionine and SAM production[98]. In an epigenome-wide association study, Lohoff et al[99] suggested that alcohol-induced hypomethylation induces hepatic SLC7A11 overexpression. Reduced *SLC7A11* methylation levels were associated with significant increases in the levels of various liver biomarkers, including gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST) alanine aminotransferase (ALT) and lipids. Similarly, Choi et al[100] found that chronic ethanol ingestion induced compensatory upregulation of SLC7A11 in hepatocyte (HEP), leading to increased Glu secretion and subsequently activated metabotropic glutamate receptor 5 on adjacent HSC to stimulate 2-arachidonoylglycerol (2-AG) production. 2-AG activated the cannabinoid type 1 receptor on HEP to generate *de novo* lipogenesis. However, some studies[9,101] have reported that SLC7A11

Table I. Potential ferroptosis-associated drugs in ALD.

Medicine	Target	Mechanism	Experimental subject	Author	Publish date
Quercetin	Iron metabolism	Alleviates the disorder of bound iron and "free" iron and regulates the BMP6/SMAD4/hepcidin signaling pathway.	Mouse	Tang et al[16,103]	2014
Fucoxanthin	Nrf2 TLR4	Upregulates the Nrf2 signal pathway and downregulates the TLR4-NF-κB signal pathway.	Mouse	Zheng et al[104]	2019
Ferrostatin-1	PUFAs	Inhibits iron-dependent lipid peroxidation.	Mouse	Liu et al[9]	2020
DMF	Nrf2	Upregulates the Nrf2-GPX4 signal pathway.	HepG2 cell and mouse	Zhang et al[105]	2020
Aronia melanocarpa	Nrf2	Regulates the PI3K/Akt/Nrf2 and Keap1/Nrf2 signal pathways.	Mouse	Wang et al[106]	2020
PFP-1	Nrf2 TLR4	Activates the Nrf2-HO-1 signal pathway and downregulates the TLR4-NF-κB signal pathway.	Mouse	Song et al[107]	2021
CQ 3MA (autophagy inhibitor)	P62	Activates the p62-Keap1-Nrf2 pathway and upregulates the expression of FTH, FPN, and HO-1.	HepG2 cell	Zhao et al[108]	2021
Fucoidan	Nrf2 Hepcidin	Enhances the p62-Nrf2-keap1-SLC7A11 signal pathway and regulates the hepatic hepcidin/intestinal DMT1/FPN1 axis.	Mouse	Xue et al[101]	2022
Melatonin	Nrf2 BMAL1	Activates the Nrf2-ARE pathway and reprograms the circadian protein BMAL1.	HepG2 cell and mouse	Zhao et al[66]	2022
Silibinin	Ferritinophagy Mitophagy	Inhibits NCOA4-dependent autophagic degradation of Ft and promotes PINK1-Parkin mitophagy.	HepG2 cells and HL7702 cells	Song et al[109]	2022

TLR4, toll-like receptor 4; BMP6, bone morphogenetic protein 6; SMAD4, SMAD family member 4; Nrf2, Nuclear factor erythroid2-related factor 2; NF-κB, nuclear factor kappa-B; PUFAs, Polyunsaturated fatty acids; DMF, Dimethyl fumarate; PI3K, Phosphatidylinositol-3-kinase; Akt/PKB, protein kinase B; Keap1, Kelch-like ECH- associated protein l; PFP-1, a polysaccharide isolated from the fruiting body of Pleurotus geesteranus; CQ, chloroquine; 3-MA, 3-Methyladenine; FTH, Ferritin Heavy Chain; FPN, ferroportin, HO-1, Heme Oxygenase-1; p62, prostacyclin; SLC7A11, Solute Carrier Family 7 Member 11; DMT1, divalentmetal-iontransporter-1; ARE, anti-oxidant response elements; BMAL1, Basic Helix-Loop-Helix ARNT Like 1; NCOA4, Nuclear receptor coactivator 4; PINK1, PTEN Induced Kinase 1.

expression in the liver is suppressed in ALD. This inconsistency may be due to differences in experimental subjects, the duration of alcohol intake, or other related mechanisms, and the crosstalk between epigenetic changes and ferroptosis in ALD requires further investigation.

Potential Drug Targets for Ferroptosis in ALD

Anti-lipid Peroxidation

Several studies[8,9] have demonstrated the efficacy of ferrostatin-1 in preventing liver injury by inhibiting iron-dependent lipid peroxidation in ALD without it being consumed. N-acetyl cysteine (NAC), a cytosine prodrug that targets SLC7A11/xCT, has shown promise in alleviating alcoholic liver injury through its effects on glutamatergic transmission (GLT-1 or Cys-Glu exchange), inflammatory pathways, oxidative stress, and GSH metabolism in advanced basic and clinical studies[102] (Table I).

Nrf2

Cheng et al[109] found that 1,25(OH)2D3 increases GPX4 activity by regulating the Keap1-Nrf2-GPX4 signaling pathway, thereby inhibiting

ferroptosis in zebrafish liver cells. Empagliflozin (EMPA), a sodium-glucose cotransporter 2 (SGLT-2) inhibitor, reduces oxidative stress and inflammation[110,111]. In a mouse model[112] of ALD, EMPA therapy elevated Nrf2 in the liver, which subsequently boosted the expression of GSH-related genes. Zhang et al[105] used a systematic computational approach to identify dimethyl fumarate (DMF), an Nrf2 inducer that remarkably reduced ROS levels, lipid peroxidation, and ferroptosis. TLR-induced signaling pathways play an opposing role to Nrf2 and are the primary inflammatory pathways in alcohol-induced liver injury[113]. Polysaccharides, such as PFP-1 and fucoxanthin, not only upregulate the Nrf2 signaling pathways but also downregulate the TLR4-NF-κB signaling pathways, thereby enhancing antioxidant defense and suppressing the inflammation in ALD[104,107].

Iron Metabolism

Tang et al[16] found that quercetin inhibits the abnormal overexpression of transferrin receptor 1 (TfR1), Ft, and metal-ion transporters induced by ethanol stimulation. They conducted further research on the mechanism of its anti-iron dyshomeostasis and found that quercetin restored hepcidin levels by reducing alcohol-induced downregulation of the bone morphogenetic protein 6 (BMP6)/SMAD family member 4 (SMAD4) signaling pathway[103]. Similarly, adding iron-chelating epigallocatechin-3-gallate (EGCG) to the diet of mice with ALD considerably improved liver damage in ALD mice and increased hepcidin mRNA transcript levels in the liver tissue[114]. Adding vitamin C to the diet of mice with ALD increases the expression of hepcidin in the mouse liver while decreasing the expression of TfR1, FPN1, and divalentmetal-iontransporter-1 (DMT1)[115]. Moreover, vitamin C can protect the liver from hepatotoxicity caused by alcohol abuse[116]. According to Xue et al[101], fucoidan reversed the decrease in hepcidin level caused by long-term alcohol exposure, reduced the liver iron load by regulating hepcidin-intestinal DMT1/FPN1, and upregulated the expression of p62/Nrf2 and SLC7A11/GPX4. This inhibited ferroptosis in hepatocytes and attenuated hepatic peroxidative damage.

Autophagy

Autophagy has emerged as a remarkable factor in ALD treatment[117]. Autophagy inhibitors, such as CQ and 3MA, protect against alcohol-induced ferroptosis by activating the p62-Keap1-Nrf2 pathway[108]. Song et al[64] discovered that silibinin reversed excessive ferritinophagy and repressed mitophagy, which promoted ferroptosis in two hepatocyte lines (HepG2 and HL7702 cells) treated with ethanol and acetaldehyde, respectively.

Conclusions

Ever since its discovery and definition by Professor Dixon and his team[1], the significance of ferroptosis in the onset and progression of numerous diseases has been established, and research on liver diseases has also attracted considerable interest. Recent studies[8,53,64] have highlighted the critical role of ferroptosis in the emergence of ALD, affecting the development of alcoholic liver injury, AFLD, ASH, and hepatocellular carcinoma. Therefore, it is conceivable that the pathophysiology of ALD and ferroptosis are inextricably linked. However, investigation into the mechanism of ferroptosis in ALD is still in its early stages, and there are still many uncharted territories to be explored. For instance, the crosstalk between epigenetic alterations and ferroptosis in ALD requires further investigation. Noncoding RNA is strongly associated with both ferroptosis and ALD. However, only a few published studies have explored their interaction in the context of ALD. Moreover, the conflicting relationships between ferroptosis, autophagy, and alcohol suggest that more detailed studies on ferroptosis and autophagy, especially for one specific type of autophagy, are required. Furthermore, the effects of many other types of autophagy on ALD are yet to be discovered, and this may provide new diagnostic and therapeutic strategies in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contributions

JB and LL conceived and designed the study, JB, ZG, and YH contributed in the acquisition of data, analysis and interpretation. The experiments were reviewed by LL, who also edited the manuscript. The submission and publication of this article were approved by all authors.

Data Availability
The datasets for this study can be found in the Pubmed (https://pubmed.ncbi.nlm.nih.gov) and Web of Science (https://www.webofscience.com/wos).

Availability of Data and Materials
The data supporting this study's findings are available from the corresponding author [E.H], upon reasonable request.

Informed Consent
Not applicable.

Ethics Approval
Not applicable.

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