Advances in Research on Ferroptosis and Inflammatory Bowel Disease: Postprint

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Abstract

Inflammatory bowel disease (IBD) represents a group of chronic nonspecific inflammatory disorders of the gastrointestinal tract, with etiology and pathogenesis potentially associated with environmental factors, genetic susceptibility, intestinal microbiota, and immune responses. Ferroptosis, a recently discovered form of iron-dependent cell death resulting from the accumulation of lipid hydroperoxides, is tightly regulated by a lipid repair system comprising glutathione (GSH) and glutathione peroxidase 4 (GPx4). Studies have demonstrated that injured intestinal tissue in IBD patients may exhibit fundamental characteristics of ferroptosis, including iron deposition, GSH depletion, GPx4 inactivation, and lipid peroxidation (LPO). Moreover, manipulation of key ferroptosis-regulating genes can modify the progression, severity, and even incidence of IBD. This review summarizes the fundamental mechanisms of ferroptosis and provides an overview of recent research perspectives on ferroptosis-related signaling pathways in IBD, offering novel directions for future clinical management of IBD.

Full Text

Preamble

Research Progress of Ferroptosis and Inflammatory Bowel Disease

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Abstract Inflammatory bowel disease (IBD) is a group of chronic non-specific inflammatory conditions of the gastrointestinal tract, whose pathogenic factors and pathogenesis may be related to environmental factors, susceptibility genes, gut microbiota, and immune response. Ferroptosis, a newly discovered form of cell death, is typically accompanied by iron accumulation and lipid peroxidation and is tightly regulated by a lipid repair system including glutathione (GSH) and glutathione peroxidase 4 (GPx4). Increasing studies have revealed that the fundamental features of ferroptosis, including iron deposition, GSH exhaustion, GPx4 inactivation, and lipid peroxidation, are manifested in the injured gastrointestinal tract of IBD patients. Furthermore, manipulation of critical ferroptotic genes could alter the progression, severity, or even morbidity of IBD. This review summarizes the basic mechanisms of ferroptosis and recent research prospects regarding ferroptosis-related signaling pathways in IBD, providing new directions for future clinical treatment of IBD.

[**Key words**] Inflammatory bowel disease; Ferroptosis; ROS; Lipid Peroxidation

1 Overview of Ferroptosis

Ferroptosis is a regulated form of novel cell death characterized by the accumulation of reactive oxygen species (ROS) and formation of lipid peroxides (LPO). Research has shown that oxygen free radicals generated by ROS more readily attack macromolecular compounds containing multiple unsaturated bonds, such as polyunsaturated fatty acids (PUFAs), thereby inducing LPO[5]. Two decomposition products of LPO—4-hydroxynonenal (4-HNE) and malondialdehyde (MDA)—cause abnormal covalent modifications of proteins and nucleic acids in the cell membrane, destroying the stability of the lipid bilayer and leading to membrane disintegration, which initiates the ferroptotic program[4-6]. Excessive ROS primarily originates from two sources: divalent iron in the intracellular labile iron pool (LIP) generates ROS through the Fenton reaction or lipoxygenases (LOXs), and the GSH/GPx4 antioxidant defense system becomes inactivated or overwhelmed. Additionally, PUFAs on the cell membrane can also undergo LPO under the action of related enzymes[5].

Ferroptosis is an iron-dependent process. Iron exists in two oxidation states: ferrous (Fe^{2+}) and ferric (Fe^{3+}). Dietary iron is reduced to Fe^{2+} in the intestine before entering intestinal mucosal epithelial cells. Fe^{2+} in the blood is oxidized to Fe^{3+} by ceruloplasmin, which then binds to serum transferrin (TF). Through the action of STEAP3 metalloreductase and divalent metal transporter 1 (DMT1), Fe^{3+} is reduced to Fe^{2+} and stored in the labile iron pool before being released into the cytoplasm[7]. Fe^{2+} is stored in ferritin complexes composed of ferritin light chain (FTL) and ferritin heavy chain 1 (FTH1), while excess Fe^{2+} is exported from cells via ferroportin 1 (Fpn1) after oxidation to Fe^{3+}, participating in systemic iron recycling to maintain intracellular iron homeostasis. Intracellular free Fe^{2+} serves as a cofactor for LOXs, which are central to fer-

roptosis[6]. Dysfunction of key iron metabolism proteins or disruption of iron homeostasis causes ROS accumulation, leading to ferroptosis. GPx4 reduces the toxicity of lipid hydroperoxides (L-OOH) by converting them to non-toxic lipid alcohols (L-OH), thereby protecting the lipid bilayer and preventing ferroptosis[8]. During this process, NADPH acts as an electron donor, so detection of NADPH abundance can be used to predict ferroptosis occurrence[9]. As a cofactor for GPx4, GSH prevents ROS accumulation, and GSH deficiency can indirectly inhibit GPx4 function[10]. The cystine/glutamate antiporter (Xc^-) is a heterodimer composed of subunits SLC7A11 and SLC3A2 linked by disulfide bonds, mediating a 1:1 exchange of intracellular glutamate for extracellular cystine. Intracellular cystine is rapidly converted to cysteine, a precursor for GSH synthesis[11]. Overall, inhibiting GSH depletion or supplementing GSH content to enhance GPx4 activity can suppress ferroptosis.

Research has found that ferroptosis sensitivity is closely related to lipid metabolism imbalance. Free PUFAs must be esterified into membrane phospholipids and undergo peroxidation to transmit ferroptosis signals. Lipidomics studies have shown that phosphatidylethanolamines (PEs) containing arachidonic acid (AA) and adrenic acid (AdA) are the key phospholipids that become oxidized and cause ferroptosis[5]. The biosynthesis and remodeling of PUFAs in the cell membrane require long-chain acyl-CoA synthetase family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3). ACSL4 converts AA and AdA into arachidonoyl-CoA (AA-CoA) and adrenoyl-CoA (AdA-CoA), which then participate in the synthesis of membrane phospholipids such as PEs through LPCAT3. These synthesized long-chain PUFAs on the membrane can be oxidized by LOXs catalyzed by Fe^{2+} to generate harmful PE-AA-OOH and PE-AdA-OOH, thereby inducing ferroptosis. Therefore, ACSL4 and LPCAT3 are considered key enzymes regulating ferroptosis[12].

Additionally, ferroptosis suppressor protein 1 (FSP1, also known as AIFM2) primarily localizes to the plasma membrane, where it acts as an oxidoreductase to reduce ubiquinone (CoQ10) to ubiquinol (CoQ10H2) using NAD(P)H. CoQ10H2 functions as a lipophilic free radical-trapping antioxidant to counteract LPO radicals and inhibit ferroptosis[13]. Recent studies have shown that GTP cyclohydrolase-1 (GCH1) is an effective ferroptosis inhibitor that mediates resistance to ferroptosis. GCH1 is the rate-limiting enzyme involved in tetrahydrobiopterin (BH4) production, and BH4 acts as a membrane antioxidant to prevent ferroptosis[9]. The GCH1-BH4 axis controls LPO by increasing antioxidant BH4 production and reducing CoQ10 abundance.

Increasing evidence demonstrates that features of ferroptosis—including iron accumulation, decreased levels of GSH and GPx4, and LPO—also appear in cancer, degenerative diseases, and ischemia-reperfusion tissues of the heart, kidney, and liver, indicating that ferroptosis participates in the pathogenesis of various diseases[14,15]. Recently, numerous studies have shown that ferroptosis is closely related to the occurrence and development of IBD.

SLC7A11, SLC3A2: constituent subunits; NADPH: reduced nicoti-

namide adenine dinucleotide phosphate; $NADP^+$: nicotinamide adenine dinucleotide phosphate; GCH1/BH4: GTP cyclohydrolase-1/tetrahydrobiopterin

[Figure 1: see original paper] Cellular Ferroptosis Pathway

2 Ferroptosis and IBD

The intestinal mucosal barrier plays an important role in IBD pathogenesis[16]. Studies have found that intestinal mucosal epithelial cells in UC patients and experimental colitis mice exhibit morphological changes characteristic of ferroptosis, including mitochondrial shrinkage and reduced mitochondrial cristae. Concurrently, the ferroptosis biomarker PTGS2 increases in intestinal epithelial cells[17], while LPO markers ROS, COX2, and ACSL4 are highly expressed at tissue, mRNA, and protein levels[18-20], and superoxide dismutase (SOD), which inhibits ROS production, is lowly expressed[20].

As previously described, iron accumulation, GSH depletion, GPx4 inactivation, and LPO are fundamental features of ferroptosis, all of which are observed in intestinal epithelial cells of IBD patients and mouse models. Ferroptosis metabolic pathways are divided into extrinsic (transporter-dependent) and intrinsic (enzyme-regulated) pathways. Recent research indicates that ferroptosis influences IBD progression through both extrinsic pathways (iron metabolism, amino acid-GSH/GPx4) and intrinsic pathways (endoplasmic reticulum stress, Nrf2/HO-1 signaling pathway, AKT/IKK/P65 and ERK/IKK/P65 signaling cascades, etc.).

2.1.1 Iron Metabolism

Extracellular Fe^{3+} enters cells and is converted to Fe^{2+}, which generates excessive ROS through the Fenton reaction or LOXs, triggering ferroptosis. Studies have found increased iron content in intestinal tissues of IBD patients, with Fe^{2+}—which plays an important role in the Fenton reaction of ferroptosis—showing the highest levels. Both mRNA and protein levels of FTL and FTH1 are significantly elevated, with FTH1-positive signals primarily observed in intestinal epithelial cells, suggesting that ferroptosis mainly occurs in epithelial cells. Additionally, research shows that deferoxamine (DFO) can chelate excess free iron to reduce ferroptosis and counteract colitis[17].

Hereditary hemochromatosis is characterized by recessive mutations in the hemochromatosis gene (Hfe). In Hfe-knockout mouse models, MDA is elevated in colon tissues, indicating that iron overload promotes oxidative damage in intestinal cells. Concurrently, mice exhibit colonic mucosal injury and are more susceptible to experimental colitis, suggesting that iron overload plays an important role in colitis pathogenesis. Iron overload-induced ROS accumulation and subsequent ferroptosis may be a pathogenic mechanism of colitis[21].

Clinically, iron deficiency is the most common cause of anemia in IBD patients, and oral iron supplementation is a routine treatment for iron deficiency anemia[22]. However, animal studies have found that oral iron supplementation may alter gut microbiota composition and metabolic processes, exacerbating intestinal inflammation[23,24]. A clinical study divided subjects into low, medium, and high iron intake groups (2.99 and 3.6 mg/4184 kJ as cutoff values) and found that higher iron intake was associated with increased odds ratios for UC incidence, indicating elevated disease risk[25]. Inappropriate iron supplementation methods or excessive iron intake typically cause ROS accumulation through Fenton and Haber-Weiss reactions, triggering oxidative stress and LPO, damaging or killing intestinal epithelial cells, and destroying intestinal mucosal barrier function. Therefore, intravenous iron injection is recommended as initial therapy for patients with active UC, severe anemia, and oral iron intolerance. Glycyrrhizin exhibits antioxidant and anti-inflammatory activities and shows promise as an effective anti-IBD agent[26]. Recent studies have found that in colitis mice, glycyrrhizin supplementation upregulates ferritin expression, increases cellular iron storage, reduces cellular iron levels, and further inhibits ferroptosis in colonic epithelial cells[27]. Currently, research on how iron overload exacerbates intestinal mucosal injury and inflammation through ferroptosis remains limited. Exploring the relationship between iron metabolism and IBD and identifying related targets may provide new directions and strategies for regulating ferroptosis and alleviating intestinal mucosal injury.

2.1.2 GSH/GPx4

The GSH/GPx4 system plays an important role in reducing lipid peroxide toxicity and preventing ferroptosis. Intestinal epithelial cells in both UC and CD patients during active disease phases exhibit reduced GPx4 activity, suggesting a close relationship between IBD and ferroptosis[17,28]. Further studies have found that in intestinal epithelial cells with reduced or deficient GPx4, ACSL4 induces the release of interleukin-6 (IL-6) and chemokine (C-X-C motif) ligand 1 (CXCL1) by regulating PUFAs, particularly AA, thereby triggering inflammation. Additionally, ACSL4 limits the production of anti-inflammatory AA metabolites such as epoxyeicosatrienoic acids (EETs). In animal experiments, GPx4-deficient mice are more susceptible to colitis than wild-type mice, highlighting the crucial role of GPx4 in protecting the intestine from LPO damage and maintaining intestinal homeostasis[28].

Recent studies have identified an important role for Paneth cells in this process[29]. Curculigoside (Cur) has been shown to promote GPx4 expression by increasing selenium sensitivity in intestinal epithelial cells, thereby alleviating histological damage in DSS-induced UC mice[18]. Similarly, clinical studies indicate that appropriate selenium supplementation in selenium-deficient populations can enhance GPx4 activity, protect cells from ferroptosis, and prevent IBD occurrence[30]. The ferroptosis inhibitor Liproxstatin-1 (Lip-1) inhibits LPO, increases GSH and FSP1 concentrations, and restores GPx4 to normal

levels, thereby enhancing the anti-ferroptosis system[31]. It has also been proven effective in improving symptoms in colitis patients and DSS-induced mouse colitis[19]. Recent research shows that the traditional Chinese medicine formula Shaoyao Decoction alleviates colitis, suppresses inflammation, and restores intestinal epithelial barrier function by activating GPx4 and inhibiting ferroptosis in colonic epithelial cells, providing scientific evidence for the clinical efficacy of Chinese herbal formulas in treating IBD[32].

Moreover, accumulating evidence demonstrates that Nrf2 participates in ferroptosis by regulating the expression of antioxidant response elements including GPx4[33]. In DSS-induced colitis mice, the Nrf2-GPx4 signaling pathway is downregulated in intestinal epithelial cells, promoting ferroptosis. The Furin protease can protect intestinal epithelial cells by activating the Nrf2-GPx4 signaling pathway to inhibit ferroptosis[34,35]. Studies have found that sulfasalazine (SAS) induces ferroptosis by inhibiting the activity of the Xc^- heterodimer that transports GSH synthesis precursors[36]. SAS is a conventional drug for IBD treatment that reduces inflammation by affecting prostaglandin synthesis. Further animal and clinical studies exploring the effect of SAS concentration on intestinal epithelial cells in IBD and balancing the relationship between inflammation inhibition and ferroptosis could help improve drug efficacy.

2.2.1 Endoplasmic Reticulum Stress

Studies have shown that endoplasmic reticulum (ER) stress not only promotes UC development but also participates in ferroptosis[37]. Protein kinase R-like ER kinase (PERK) is the primary sensor of ER stress. RSL3 is an inhibitor of GPx4, and research has found that the PERK inhibitor GSK414 not only suppresses the RSL3-induced ER stress signaling pathway eIF2α/ATF4/CHOP but also reduces ferroptosis, thereby improving experimental colitis in mice. This indicates that ferroptosis regulates UC through ER stress-mediated intestinal epithelial cell death[17]. Further studies have revealed that phosphorylated NF- B p65 interacts with its regulatory factor eIF2α to inhibit ER stress-mediated ferroptosis in intestinal epithelial cells[38], suggesting that NF- B p65 may be a potential therapeutic target for UC.

2.2.2 Nrf2/HO-1 Signaling Pathway

Nrf2 not only protects intestinal epithelial cells by inhibiting ferroptosis through the Nrf2-GPx4 signaling pathway but also promotes ferroptosis via the Nrf2/HO-1 pathway. On one hand, Nrf2 and HO-1 are significantly upregulated in mouse colitis, exerting anti-inflammatory and antioxidant effects[19,39]. Astragalus polysaccharide (APS) can prevent ferroptosis in mouse colitis and human Caco-2 cells by inhibiting this signaling pathway[40], suggesting that ferroptosis may regulate DSS-induced UC through the Nrf2/HO-1 pathway. On the other hand, excessive activation of Nrf2/HO-1 leads to ferroptosis by disrupting iron ion metabolism balance[41,42]. Ferrostatin-1 (Fer-1) can inhibit ferroptosis and

improve symptoms in colitis patients and DSS-induced mouse colitis by down-regulating Nrf2/HO-1 expression and chelating Fe^{2+} in the labile iron pool to reduce free iron concentration[19,43]. Currently, the specific mechanisms linking the Nrf2/HO-1 signaling pathway with ferroptosis remain unclear and require further investigation.

2.2.3 AKT/IKK/P65 and ERK/IKK/P65 Signaling Cascades

Maternal embryonic leucine zipper kinase (MELK) regulates cell proliferation, apoptosis, and differentiation, affecting stem cell phenotype and tumorigenesis[44]. Studies have found that MELK expression is elevated in both colitis patients and mouse models compared to normal controls. The MELK inhibitor OTSSP167 protects colitis mouse intestinal tissue by maintaining normal gut microbiota composition, balancing microbial distribution, inhibiting ferroptosis in intestinal epithelial cells, reducing pro-inflammatory factor expression in intestinal tissue, and suppressing the AKT/IKK/P65 and ERK/IKK/P65 signaling cascades in intestinal tissues both in vivo and in vitro[45]. Ferroptosis may regulate intestinal epithelial cells in IBD through phosphorylated AKT, ERK, IKK, and P65, providing new perspectives for IBD treatment, and MELK may be a potential therapeutic target.

2.3 Other Factors

As previously mentioned, LPO of PUFAs on the cell membrane leads to ferroptosis. Previous studies have suggested that the increased incidence of IBD is synchronized with increased dietary PUFA (such as AA) intake[46]. Large prospective clinical trials in CD patients have found that PUFA supplementation may worsen IBD symptoms such as diarrhea, indicating disturbed intestinal homeostasis[47]. α-Tocopherol, the most active form of vitamin E hydrolysis products, can prevent PUFA-induced LPO, cytokine production, and neutrophil infiltration, thereby inhibiting ferroptosis to some extent[28]. Additionally, direct dietary supplementation with monounsaturated fatty acids (MUFAs) to replace PUFAs susceptible to LPO in the cell membrane can prevent lipid ROS accumulation and ferroptosis[48]. Moreover, CoQ10H2 acts as a lipophilic free radical-trapping antioxidant to counteract LPO radicals and inhibit ferroptosis. A recent randomized controlled trial found that CoQ10 supplementation, which can be reduced to CoQ10H2 by FSP1, effectively alleviates inflammation in remission-stage mild-to-moderate UC patients[49]. Currently, direct clinical studies on ferroptosis and IBD remain limited, but a series of studies have proposed the hypothesis that a more balanced diet (with balanced iron, selenium, CoQ10, and fatty acids) may be a better choice for improving IBD symptoms, maintaining gastrointestinal health, and preventing IBD occurrence. These viewpoints still require extensive animal experiments and clinical studies for verification.

OTSSP167: Type I kinase inhibitor; PERK: protein kinase R-like ER kinase; ACSL4: long-chain acyl-CoA synthetase family member

4; Lip-1: ferroptosis inhibitor; Furin: endoprotease

3 Summary and Outlook

[Figure 2: see original paper] Ferroptosis Regulatory Pathways in IBD Cells

Ferroptosis, as a newly discovered cell death modality, is currently a research hotspot, with numerous studies demonstrating its close relationship with IBD. This review summarizes the potential signaling pathways through which ferroptosis regulates IBD. By exploring the mechanisms of ferroptosis and related targets, we can regulate ferroptosis occurrence and effectively alleviate IBD progression in experimental animals. However, many questions remain to be addressed: What are the specific mechanisms of intrinsic metabolic pathways of ferroptosis in IBD? How can iron intake dosage be controlled? Since ROS widely exists in numerous cells, how can we intervene specifically? In addition to intestinal epithelial cells, do intestinal immune cells also undergo ferroptosis? Currently, IBD pharmacotherapy primarily focuses on immunosuppression, which cannot completely resolve enteritis occurrence. Therefore, further in-depth research to elucidate the specific mechanisms and regulatory factors of ferroptosis is expected to provide new therapeutic targets for IBD.

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Note: Figure translations are in progress. See original paper for figures.