Role of ferroptosis in the pathogenesis and as a therapeutic target of inflammatory bowel disease (Review)

DICKSON KOFI WIREDU OCANSEY[1,2*], JINTAO YUAN[3*], ZHIPING WEI[1], FEI MAO[1] and ZHAOYANG ZHANG[4]

[1]Key Laboratory of Medical Science and Laboratory Medicine of Jiangsu Province, School of Medicine, School of Materials Science and Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, P.R. China; [2]Directorate of University Health Services, University of Cape Coast, Cape Coast CC0959347, Ghana; [3]The People's Hospital of Danyang, Affiliated Danyang Hospital of Nantong University, Zhenjiang, Jiangsu 212300; [4]Clinical Laboratory, Taicang Hospital of Traditional Chinese Medicine, Suzhou, Jiangsu 215400, P.R. China

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Abstract. Ferroptosis, a novel form of regulated cell death, is characterized by the accumulation of labile iron and lipid peroxidation, and the excessive production of reactive oxygen species (ROS). Although ferroptosis lies at the center of crucial biological activities involving O_2, iron and polyunsaturated fatty acids (PUFAs), which are essential for cell proliferation and growth, the interaction between these molecules could also mediate the accumulation of toxic levels of ROS and lipid peroxides, which can then cause damage to cellular membranes and ultimately result in cell death. Recent reports have indicated that ferroptosis participates in the development and progression of inflammatory bowel disease (IBD), offering a new exploratory field which may aid in the more in-depth understanding of the pathogenesis and therapeutic targets of IBD. Of note, the mitigation of the characteristic features of ferroptosis, such as depleted glutathione (GSH) levels, inactivated glutathione peroxidase 4 (GPX4), elevated levels of lipid peroxidation and iron overload significantly relieve IBD. This has attracted the attention of researches aiming to examine therapeutic agents that inhibit ferroptosis in IBD, including radical-trapping antioxidants, enzyme inhibitors, iron chelators, protein degradation inhibitors, stem cell-derived exosomes and oral N-acetylcysteine or glutathione. The present review summarizes and discusses the current data that implicate ferroptosis in the pathogenesis of IBD and its inhibition as a novel alternate therapeutic target for IBD. The mechanisms and key mediators of ferroptosis, including GSH/GPX4, PUFAs, iron and organic peroxides are also discussed. Although the field is relatively new, the therapeutic regulation of ferroptosis has exhibited promising outcomes as a novel treatment avenue for IBD.

Correspondence to: Dr Zhaoyang Zhang, Clinical Laboratory, Taicang Hospital of Traditional Chinese Medicine, 140 Renmin South Road, Taicangy, Suzhou, Jiangsu 215400, P.R. China
E-mail: zhangcy19791216@163.com

*Contributed equally

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Contents

1. Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder that affects the gastrointestinal tract, specifically the colon and/or the small intestine. IBD encompasses two main types of conditions: Crohn's disease (CD) and ulcerative colitis (UC). IBD can have a significant impact on a the quality of life of affected individuals, and the condition requires lifelong management with medications, lifestyle changes and sometimes even surgery (1). Current medical treatments for IBD focus on the use of drugs, such as corticosteroids, aminosalicylates, immunomodulators and biologics to manage symptoms, alongside other measures or surgery if deemed necessary. However, a significant number of patients either do not respond to these treatments or eventually, these lose their effectiveness, indicating a need for alternative therapies (2). Various new treatment options are being explored, such as the use of small molecules, apheresis therapy, enhancing gut microecology, cell therapy and exosome therapy (3,4). The exact cause of IBD is not yet fully understood, although it is considered to occur due to a complex interplay between genetic, environmental and immune system factors. These contribute to the activation of

various regulated cell death mechanisms, including ferroptosis, which has been implicated in the pathogenesis of IBD (5,6).

Since its discovery in 2012 (7), ferroptosis has gained the attention of researches worldwide and has been studied across several diseases (8-10). In contrast to other types of cell death, such as apoptosis, necroptosis and autophagy, ferroptosis exhibits unique sets of biological processes and pathophysiological characteristics and is mainly accompanied by iron accumulation and lipid peroxidation (11,12). In other words, the execution machinery of ferroptosis is comprised of the availability of redox-active iron, peroxidation of polyunsaturated fatty acid-(PUFA) containing phospholipids (PLs), and the inactivation of the lipid peroxide repair system (13). Distinctively, the cytological characteristic hallmarks of apoptotic cell death, including chromatin condensation and plasma membrane blebbing, as well as the morphological characteristics of necroptosis, i.e., the swelling of cytoplasmic organelles, are rarely observed during ferroptotic cell death (7,13). Approximately 10 years following its discovery, substantial progress has been made concerning studies on ferroptosis, including the characterization of core molecular marks and regulatory components, such as glutathione peroxidase (GPX)4, solute carrier family 7 member 11 (SLC7A11), ferroptosis suppressor protein 1 (FSP1, also known as AIFM2), nuclear factor erythroid 2-related factor 2 (Nrf2) and p53, the identification of inducers, such as erastin, FINO2, RAS-selective lethal 3 (RSL3) and FIN56, and specific inhibitors ferrostatin-1, liproxstatin-1 and deferoxamine, which have together strengthened the fundamental understanding of ferroptotic cell death (11,12).

Current evidence implicates ferroptosis in gastrointestinal conditions, such as IBD and its associated colorectal cancer (12,14), presenting it as a potential novel therapeutic target. The pathological engagement of ferroptosis has been demonstrated in both patients with IBD and experimental models, including elevated iron deposition, glutathione (GSH) exhaustion, GPX4 inactivation and lipid peroxidation, which together contribute to intestinal cell death and sustained inflammation (5,11,12,15). These factors further drive inflammation and upregulate ferroptosis, exacerbating intestinal tissue and barrier injury (16). A number of studies have demonstrated that the inhibition of ferroptosis significantly mitigates the characteristic clinical features of IBD, including improved intestinal barrier function, body weight, tissue repair, anti-inflammation, microbiota and a decreased disease activity index (15,17-19). These findings present not only a novel understanding of the pathogenesis of IBD, but may also aid in the development of therapeutic approaches for IBD and other intestinal diseases. The present review comprehensively summarizes and discusses the role of ferroptosis in the pathology of IBD and explores the currently available data on therapeutic targets of ferroptosis in IBD. The mechanisms and key mediators of ferroptosis, as well as pathogenic involvement and therapeutic targets in other intestinal diseases, are also discussed.

2. The mechanisms of ferroptosis

Ferroptosis is primarily triggered by small molecules, such as erastin and the inhibition of glutathione biosynthesis or GPX4, in the presence of iron-dependent accumulation of lipid reactive oxygen species (ROS) and the depletion of plasma membrane PUFA (20). Ferroptosis can occur through two major pathways, the extrinsic or transporter-dependent pathway that includes increased iron uptake and decreased cysteine/glutamine uptake, and the intrinsic or enzyme-regulated pathway that includes the inhibition of GPX4 (21). In addition to the initial discovery of the inhibitory role of the cystine-import-GSH-GPX4 machinery in ferroptosis, the role of PL hydroperoxides (PLOOHs) as the executioners of ferroptosis has also been established. Studies on the mechanism of PLOOH synthesis, particularly, the synthesis and activation of PUFAs, the precursor of PLOOHs, have been extensively reported (22). In the inhibition of ferroptosis, GPX4 serves as the key PLOOH-neutralizing enzyme. Thus, in the canonical GPX4-regulated ferroptotic pathway, the inhibition of two cellular components, GPX4 and system X_C^- cystine/glutamate antiporter, by RSL3 and erastin, respectively, triggers ferroptotic cell death (7,23). In the exploration of the general mechanism underlying erastin/RSL3-induced ferroptosis, it has been noted that both compounds inactivate GPX4, as RSL3 directly inactivates GPX4, while erastin indirectly inactivates GPX4 by preventing the import of cystine, leading to the deprivation of cysteine, a crucial building block of GSH and cellular antioxidants. As a result, PLOOHs accumulate, leading to rapid and unrepairable plasma membrane damage, and consequently cell death (24).

A key hallmark of ferroptosis is uncontrolled lipid peroxidation. In this process, a bis-allylic hydrogen atom is abstracted from the polyunsaturated fatty acyl moieties found on PLs (PUFA-PLs) located in lipid bilayers, causing the formation of a carbon-centered radical, and subsequently reacting with molecular oxygen to produce peroxyl radicals (25). The free radicals mediate further reactions that result in the formation of a myriad of secondary products, which subsequently cause the breakdown of cell membrane integrity and ultimately rupturing of organelle and cell membranes. Therefore, the membrane-repairing enzymes, acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase (LPCAT)3 are crucial in ferroptosis. ACSL4 has the ability to ligate long-chain PUFAs with coenzyme A, whereas LPCAT enzymes re-esterify the ligated long-chain PUFAs in phospholipids (26,27). Lipoxygenases (LOXs), a group of non-heme iron-dependent dioxygenases, participate in ferroptosis by directly oxygenating PUFAs of cellular membranes; thus, their inhibition prevents ferroptosis (28). While the ferroptotic cell death processes can be reversible, the cell recovery phenomena could be mediated by different mechanisms yet to be fully clarified. Currently, there is no direct lipid peroxidation rescue experiment using ACSL4. Instead, studies have shown that the application of GSH or ferrostatin-1 can promote the reversal of ferroptosis, possibly because GSH enhances the GPX4 activity to arrest ROS accumulation (29), while ferrostatin-1 is a ROS scavenger that can remove the excessive cytosolic and lipid ROS (30).

Iron is involved in the mechanism of ferroptosis through the implication of the metabolic enzymes of phospholipid peroxidation (LOXs and POR) which require iron for catalysis, and through the iron-dependent Fenton chain reaction (a non-enzymatic reaction). In the Fenton reaction, ferrous

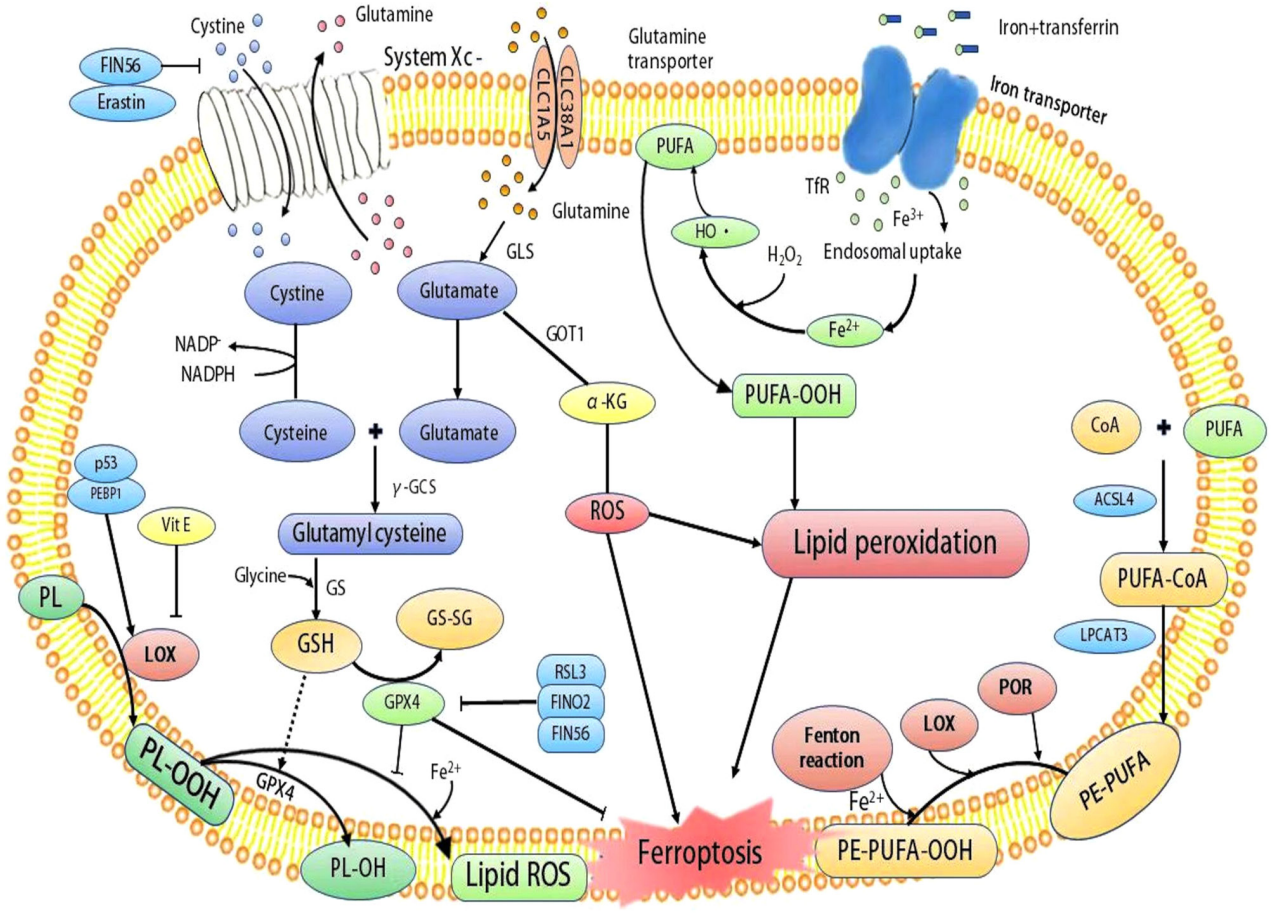

Figure 1. Mechanisms of ferroptosis. System X_C^- and glutamine transporter allow the influx of cystine and glutamine, respectively, which are converted into cysteine and glutamate. Cysteine combines with glutamate to form glutamylcysteine by γ-GCS, followed by conversion to GSH under the action of GS. GSH and GPX4 serve as scavengers of ROS to prevent oxidative stress and lipid peroxidation. However, the inhibition of system Xc- by agents, such as erastin and FIN56 depletes GSH, leading to increased ROS and lipid peroxidation. Moreover, while RSL3, FINO2 and FIN56 can directly inhibit GPX4 to promote lipid peroxidation, p53 and PEBP1 enhance the activity of LOX to promote lipid peroxidation, resulting in ferroptosis. In addition, POR, LOX and the Fenton reaction chain promote the conversion of PE-PUFA to PE-PUFA-OOH, leading to ferroptosis. GSH, glutathione; GS, GSH synthetase; γ-GCS, γ-glutamylcysteine synthetase; GPX4, glutathione peroxidase 4; PUFA, polyunsaturated fatty acid; GOT1, glutamate oxaloacetate transaminase 1; GSSG, oxidized glutathione; ROS, reactive oxygen species; LOX, lipoxygenase; POR, cytochrome P450 oxidoreductase, ACSL4, acyl-CoA synthetase long-chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; PEBP1, phosphatidylethanolamine-binding protein-1; PL, phospholipid; PLOOH, phospholipid hydroperoxides; PE, phosphatidylethanolamine; RSL3, RAS-selective lethal 3.

(Fe^{2+}) or ferric (Fe^{3+}) ions react with hydrogen peroxide (H_2O_2) to generate hydroxyl radicals, a form of ROS that triggers the peroxidation of PUFAs in membrane lipids, leading to ferroptosis (31,32). Metabolism plays a central role in ferroptosis, as several cellular metabolic reactions can result in the production of PLOOHs and autophagy (33). For example, the influence of autophagy in cystine-deprivation-induced ferroptosis is mediated through the autophagic degradation of the iron-storage protein ferritin (ferritinophagy), which leads to increased cellular labile iron content, and consequently to sensitization to ferroptosis (8). Multiple autophagy-related genes have been identified as positive regulators of ferroptosis, where the induction of ferroptosis led to autophagy activation and consequent degradation of ferritin and ferritinophagy cargo receptor nuclear receptor coactivator 4 (NCOA4). The inhibition of ferritinophagy by the knockdown of NCOA4 or the blockage of autophagy prevents the accumulation of ferroptosis-associated cellular labile iron and ROS, as well as eventual ferroptotic cell death; thus, ferroptosis is an autophagic cell death process (34). Moreover, the participation of the mitochondria in ferroptosis underscores its metabolic nature, as one of the hallmarks of ferroptosis is a specific morphological phenotype characterized by extensive ultrastructural changes of mitochondria (8). The critical role of the mitochondria in lipid peroxidation-driven ferroptotic cell death has been observed in several conditions, including excess free-iron accumulation in the mitochondria, impaired mitochondrial metabolism, resulting in the extensive production of ROS, and mitochondrial cysteine deprivation (9,35). As a major fuel of the mitochondrial TCA cycle, glucose regulates ferroptosis, since its deprivation inhibits ferroptosis via 5'AMP-activated protein kinase (AMPK) signaling (36). On the mitochondrial membrane, cytoplasmic iron activates mitoferrin 2, which in turn increases iron transport into the mitochondria, leading to elevated ROS production and ferroptosis. As previously demonstrated, mice fed an iron-deficient diet for 3 weeks exhibited a significant reversal in intestinal injury induced by abdominal ionizing radiation exposure (37). The mechanisms involved in ferroptosis and their interconnections are summarized in Fig. 1.

3. Key mediators of ferroptosis

PUFAs. The peroxidation of PUFAs by lipoxygenases is a key driver of ferroptosis. It has been demonstrated that the mechanism of lipid peroxidation during ferroptosis involves the phosphorylase kinase G2 (PHKG2) regulation of iron availability to LOX enzymes, which in turn drives ferroptosis through the peroxidation of PUFAs at the bis-allylic position. The pretreatment of cells with PUFAs that contain the heavy hydrogen isotope deuterium at the site of peroxidation (D-PUFA) inhibits PUFA oxidation and blocks ferroptosis (38). This indicates that the oxidation of PUFA by LOXs through a PHKG2-dependent iron pool is required for ferroptosis, and that the covalent inhibition of the catalytic selenocysteine in GPX4 prevents the elimination of PUFA hydroperoxides. In a multicenter case-control study on 83 patients newly diagnosed with UC, the authors found that the risk of developing UC was significantly decreased in patients who consumed n-6/n-3 PUFAs, as compared to those who consumed larger amounts of docosahexaenoic, eicosapentaenoic and docosapentaenoic acid (39). However, an earlier study reported that in addition to n-3 PUFAs, both eicosapentaenoic acid and docosahexaenoic acid confer a statistically significant protective effect against the risk of developing UC in a cohort aged >45 years (40). It has also been reported that the PUFA biosynthesis pathway determines ferroptosis sensitivity in gastric cancer, as the expression of elongation of very long-chain fatty acid protein (ELOVL)5 and fatty acid desaturase 1 (FADS1) is upregulated in mesenchymal-type cancer cells, and leads to ferroptosis sensitization (41). These findings implicate PUFAs as key mediators of ferroptosis.

GSH/GPX4. The excessive production and accumulation of ROS induce lipid peroxidation, a key mechanism of ferroptosis. GSH, the reducing substrate of GPX4 activity, is indispensable for preventing ferroptosis (42). The combined effect of GSH/GPX4 protects cells from ferroptotic death, with GPX4 serving as the cornerstone of the antiperoxidative defense. As GSH inhibits the accumulation of ROS, its depletion turns has been shown to be associated with marked lipid peroxidation, dysregulated cellular functions and cell death (43). GSH is synthesized from glycine, L-cysteine and L-glutamate, and the system X_C^- cystine/glutamate antiporter, which comprises a light-chain subunit (SLC7A11, xCT) and a heavy-chain subunit (SLC3A2, CD98hc), mainly regulates the exchange of intracellular L-glutamate and extracellular L-cystine. However, certain molecules, such as erastin and sulfasalazine can inhibit system X_C^- and cause the depletion of cellular cysteine, leading to the impairment of the intracellular GSH homeostasis and ultimately, intracellular GSH deficiency and ferroptosis (44,45). GPX4 modulates ferroptosis by preventing lipid peroxide-induced toxicity and protecting the membrane lipid bilayers through the conversion of toxic lipid hydroperoxides into non-toxic lipid alcohols (46). On the other hand, RSL3 potently drive ferroptosis by directly targeting and inhibiting GPX4 activity and promoting ROS production (14), as well as through NF-κB pathway activation and GPX4 depletion (47).

Iron. Due to the central role played by iron in cell viability and death, cellular iron homeostasis is subjected to exquisite control, mainly through a post-transcriptional network dictated by iron-regulatory protein (IRP)1 and IRP2, which modulate intracellular iron import/export and storage/release (48). Cellular iron is involved in ferroptosis through two major routes. Firstly, the metabolic enzymes implicated in the peroxidation of phospholipids, LOXs and POR, require iron for catalysis; iron is also crucial for a plethora of metabolic enzymes involved in the generation of cellular ROS. LOXs and POR can catalyze enzymatic lipid peroxidation in a Fe^{2+}-dependent manner and are implicated in the generation of PLOOH, factors necessary for ferroptosis (49). Secondly, the iron-dependent Fenton chain reaction, a non-enzymatic chemical reaction, is critical for ferroptosis; the inhibition of GPX4 causes PLOOHs to persist longer, triggering the Fenton reaction to rapidly amplify PLOOHs, the hallmark of ferroptosis (25). Moreover, PLOOHs can interact with both ferric and ferrous ions to produce the free radicals, PLOO· and PLO· respectively, which subsequently react with PUFA-PLs to further drive PLOOH production. Therefore, mechanisms that lead to improved ion homeostasis by an enhanced cellular iron export result in a more ferroptosis-resistant cell (50). A previous multicenter, hospital-based case-control study on patients newly diagnosed with UC indicated that the highest intake of iron led to an increased odds ratio for UC in a multivariate analysis, implicating that a high intake of iron has a certain effect on the development of UC (51). It has also been reported that the characteristic iron overload in hereditary hemochromatosis, causes multiple metabolic disturbances, disrupts colonic homeostasis and colon-microbiome interaction, and exacerbates the development and progression of colonic inflammation and colon cancer (52). This may be related to the involvement of excess iron in ROS generation in the intestinal mucosa, enhancing cellular damage and loss of barrier integrity.

Organic peroxides. Organic peroxides are highly reactive and thermally unstable compounds containing one or more oxygen-oxygen bonds (ROOR) and can undergo self-accelerating decomposition. In this process, the O-O linkage can easily be broken down, releasing free radicals in the form of RO· (alkoxy anions). Organic peroxides are mostly exploited in research models to generate oxidative damage in cells. For instance, tert-butyl hydroperoxide is a typical lipid peroxide analog generally known to stimulate cellular damage due to lipid peroxidation-dependent ferroptosis in human and murine cell lines (53,54). Artemisinin and its derivative compounds, such as artesunate and dihydroartemisinin are 1,2,4-trioxane-based organic peroxides, and effectively sensitize cancer cells to ferroptosis by regulating iron homeostasis (55,56). FINO2, a ferroptosis-inducing organic peroxide containing a 1,2-dioxolane skeleton, indirectly inhibits GPX4 and oxidizes iron. It decreases GPX4 activity and protein levels *in vitro*, but does not act as an active site, allosteric, or covalent inhibitor of GPX4 or alter GPX homeostasis (57). In other words, FINO2 has a dual induction mechanism for ferroptosis, involving direct iron oxide or indirect inhibition of GPX4 activity. The role of the key mediators of ferroptosis, as described above is illustrated in Fig. 2.

4. Role of ferroptosis in the pathogenesis of IBD

Studies have indicated that the fundamental characteristics of ferroptosis, including increased lipid peroxidation, the

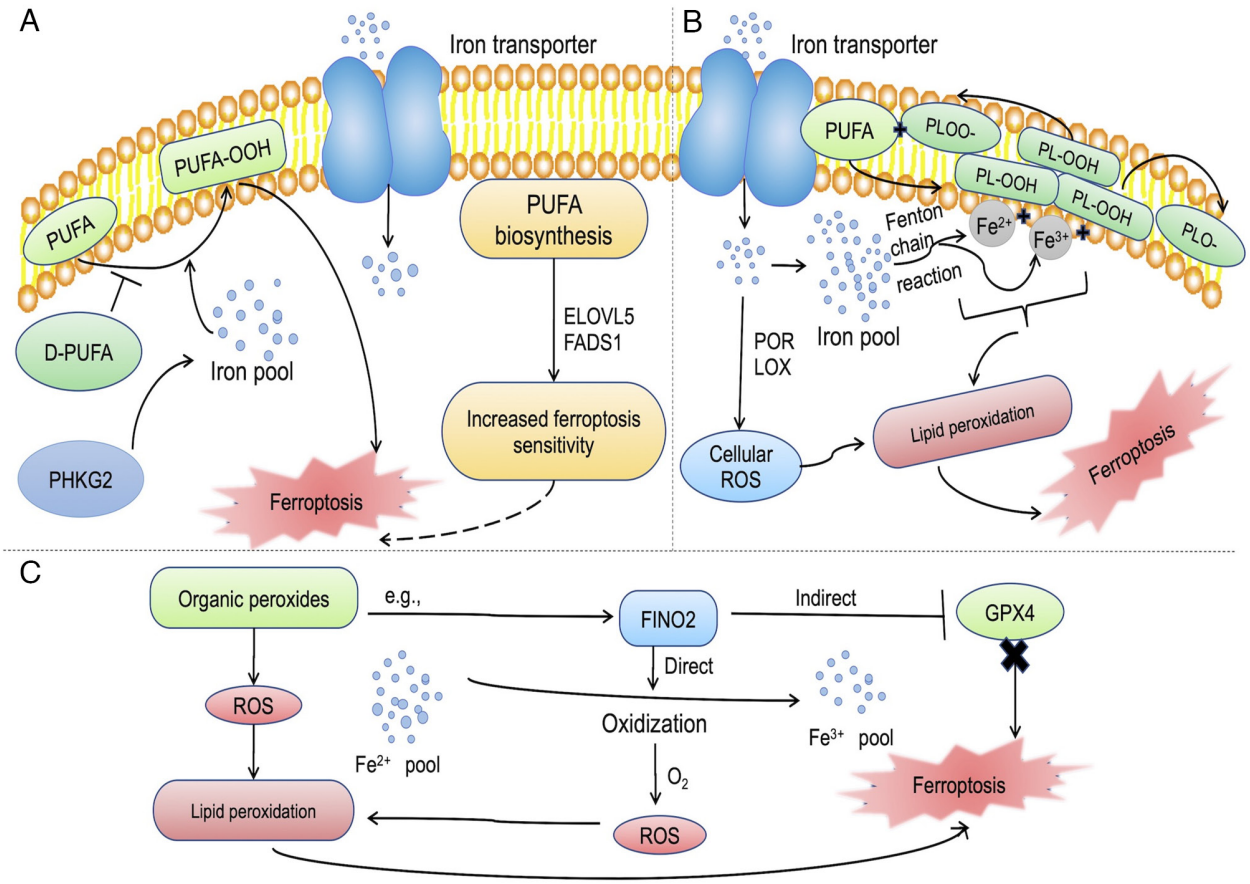

Figure 2. Role of key negative mediators of ferroptosis. (A) PUFA serves as one of the drivers of ferroptosis. The increased accumulation of cellular irons triggers the oxidation of PUFA to PUFA-OOH, which is promoted by PHKG2, leading to ferroptosis. ELOVLS and FADS1 enhance cellular sensitivity to ferroptosis, while D-PUFA inhibits the oxidation of PUFAs. (B) Iron, both Fe^{2+} and Fe^{3+}, induces lipid peroxidation through the Fenton chain reaction by interacting with PL-OOH to produce PLOO· and PLO·, respectively, and through the enzymatic route, POR and LOX, to produce cellular ROS that drives ferroptosis. (C) Organic peroxides contribute to ferroptosis by ROS production. FINO2, a typical organic peroxide drives ferroptosis by either inhibiting GPX4 or oxidizing iron to release ROS, causing lipid peroxidation and subsequently, ferroptosis. PUFA, polyunsaturated fatty acid; PUFA-OOH, polyunsaturated fatty acid containing-phospholipid hydroperoxides; PHKG2, phosphorylase kinase G2; PLOOH, phospholipid hydroperoxides; LOX, lipoxygenase; POR, cytochrome P450 oxidoreductase; ROS, reactive oxygen species; GPX4, glutathione peroxidase 4.

depletion of GSH, the inactivation of GPX4 and elevated iron deposition, are manifested in the inflamed intestinal tissue of patients with IBD and in animal models of IBD (19,58,59). Ferroptosis is involved in IBD, particularly intestinal epithelial cell death (5). As a characteristic of several intestinal diseases, such as IBD, an aberrant elevation in the rate of intestinal epithelial cell death underlies instances of extensive epithelial erosion. The mode of programmed cell death influences intestinal tissue restitution responses with implications for chronic inflammation, and ultimately the long-term risks of intestinal fibrosis and colorectal cancer (60). Ferroptosis participates in driving the chronic aberrant inflammation in IBD by a lethal accumulation of ROS, iron overload and uncontrolled lipid peroxidation, leading to intestinal epithelial cell death and epithelial erosion (15,58). Moreover, the dysregulation of critical ferroptosis-related genes has been shown to alter the progression, severity, or even morbidity of mice with experimental colitis (61,62). Compared with the control samples, colitis specimens exhibit significantly higher malondialdehyde (MDA) (58) and iron (particularly, ferrous iron) levels (63), and increased cellular iron levels during ferroptosis induce transcriptional upregulation of ferritin (34), which may contribute to the exacerbation of colitis.

A previous study found that ferroptosis is significantly induced in the intestinal epithelial cells of both patients with UC and mice with colitis, and is mediated by a number of signaling, including the endoplasmic reticulum (ER) stress signaling. In intestinal epithelial cells, the deletion of NF-κBp65 upregulates ferroptosis and exacerbates colitis, whereas phosphorylated-NF-κBp65 significantly inhibits ER stress signaling by directly binding eukaryotic translation initiation factor 2α (5). These observations indicate that ferroptosis can participate in UC through ER stress-mediated intestinal epithelial cell death, and the phosphorylation of NF-κBp65 inhibits ER stress-mediated ferroptosis in intestinal epithelial cells to alleviate UC. Genetic analysis has also revealed primary aberrance in several ER homeostasis-associated genes, including anterior gradient protein 2 homolog, X-box binding protein 1 and sphingolipid biosynthesis regulator 3 in patients with UC (64). The inflamed tissue of patients with IBD exhibits a reduced expression of aryl-hydrocarbon receptor repressor (Ahrr) and the loss of intestinal intraepithelial lymphocytes (IELs) in $Ahrr^{-/-}$ mice increases the susceptibility to dextran sulfate sodium (DSS)-induced colitis and *Clostridium difficile* infection. Mechanistically, Ahrr deficiency results in ferroptosis in $Ahrr^{-/-}$ IELs through the AHR-induced expression of

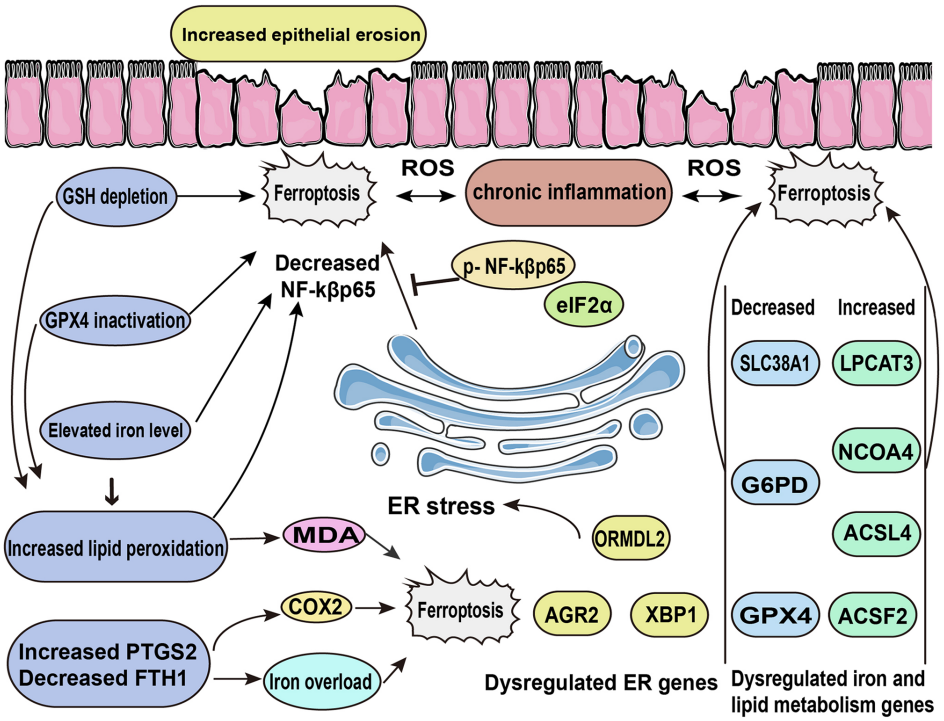

Figure 3. Role of ferroptosis in the pathology of inflammatory bowel disease. In the gut, lipid peroxidation, which is driven by depleted GSH, inactive GPX4 and elevated iron levels, leads to increased ferroptosis that consequently promotes epithelial cell erosion. Other factors, such as ER stress and dysregulated genes contribute to chronic inflammation, ROS generation and ferroptosis. GSH, glutathione; GPX4, glutathione peroxidase 4; ROS, reactive oxygen species; ER, endoplasmic reticulum, MDA, malondialdehyde; COX2, cyclooxygenase 2; eIF2α, eukaryotic translation initiation factor 2α; ORMDL2, ORMDL sphingolipid biosynthesis regulator 2; AGR2, anterior gradient protein 2 homolog; XBP1, X-box binding protein 1; SLC38A1, solute carrier family 38, member 1; G6PD, glucose-6-phosphate dehydrogenase; GPX4, glutathione peroxidase 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; NCOA4, nuclear receptor coactivator 4; ACSL4, acyl-CoA synthetase long-chain family member 4; ACSF2, acyl-CoA synthetase family member 2.

CYP1A1, a monooxygenase that generates ROS, increasing oxidative stress, redox imbalance and lipid peroxidation (65). Thus, a tight regulation that prevents oxidative stress and ferroptosis, preserves intestinal immune responses and inflammation. Moreover, it has been reported that the deficiency in GSH antioxidant function may participate in the pathogenesis of UC-related liver injury, since the reduction of hepatic GSH synthesis and reduced GSH function occurs earlier than liver injury in UC. Moreover, a significant positive correlation has been between the severity of colonic lesions and the degree of liver injury (66).

Several ferroptosis-associated genes, including *ACSL4*, acyl-CoA synthetase family member 2, *GPX4, LPCAT3*, *NCOA4*, solute carrier family 38, member 1 (*SLC38A1*) and glucose-6-phosphate dehydrogenase, all of which participate in the regulation of lipid or iron metabolism (10,67), have been shown to be markedly dysregulated (downregulated or upregulated) in UC specimens (5). In the analysis of ferroptosis-related genes in patients with UC, 26 differentially expressed genes have been identified that are significantly enriched in energy pathways and metabolism, with the top 10 hub genes from the protein-protein interaction network as interleukin 6 (*IL6*), prostaglandin-endoperoxide synthase 2 (*PTGS2*), hypoxia inducible factor 1 alpha *(HIF1A)*, cluster of differentiation 44 (*CD44)*, mucin 1 (*MUC1)*, caveolin 1 *(CAV1)*, nitric oxide synthase 2 *(NOS2)*, C-X-C motif chemokine ligand 2 *(CXCL2)*, stearoyl-CoA desaturase *(SCD)*, and acyl-CoA synthetase long chain family member 4 *(ACSL4)* (62). Moreover, *GSE94648, CD44* and *MUC1* were highly expressed and consistent with the expression trend in *GSE75214* (62). Results from animal model have also revealed the significantly increased expression of *CD44* in the colon. It was thus concluded that *CD44* and *MUC1* may be ferroptosis-related markers in UC (62), and thus, serve as new directions for UC diagnosis and treatment. In a similar study, the analysis of 97 UC and 75 CD samples alongside 22 normal controls revealed six differently expressed long non-coding RNAs (lncRNAs) associated with ferroptosis and immunity in IBD. A constructed lncRNA-mRNA regulatory network further identified potential miRNAs and transcription factors based on trans and competing endogenous RNA mechanisms, concluding that ferroptosis and immune-related lncRNA are involved in the regulation of aberrant immune response in IBD (59). The implication of ferroptosis in IBD pathology, as outlined above, is illustrated in Fig. 3.

5. Role of ferroptosis in the pathogenesis of other intestinal diseases

Research on ferroptosis continues in the quest to provide a more in-depth understanding of intestinal disease pathogenesis in order to improve clinical treatment and diagnosis. In addition to IBD, studies have implicated ferroptosis in other intestinal diseases, such as intestinal ischemia/reperfusion (I/R) injury (68), necrotizing enterocolitis (69), colitis-associated colorectal cancer (CAC) (70), and other gastrointestinal diseases, including pancreatitis (16), liver diseases (71) and gastrointestinal cancers, such as colorectal, liver and gastric cancers (72-74). The hallmark characteristics of ferroptosis,

namely ROS generation, lipid peroxidation, depleted GSH expression and increased MDA levels have been demonstrated in intestinal I/R injury (6,68). In intestinal tissues from mice the I/R model, the levels of pro-ferroptotic factors, such as iron and ACSL4 are increased, while those of anti-ferroptotic factors, such as GPX4, ferritin heavy chain 1 (FTH1) and GSH are decreased, with the inhibition of ferroptosis ameliorating intestinal injury and barrier dysfunction (68).

Ferroptosis has been implicated in the pathophysiology of tumors, including colorectal cancer (75). The role of ferroptosis regulators, such as LPCAT3, SLC7A11, erastin, GPX4 and ACSL4, among others have been demonstrated in colorectal tumors. For example, the regulation of GPX4 by SRSF9 serves as a crucial mechanism that drives colorectal cancer tumorigenesis, as well as Erastin-induced ferroptosis resistance (76), and TP53-induced glycolysis and apoptosis regulator (TIGAR) functions as a potential modulator of ferroptosis resistance in colorectal cancer through the ROS/AMPK/SCD1 signal pathway, with the knockdown of TIGAR causing elevated lipid peroxidation and sensitive to erastin-induced ferroptosis (77). The induction of ferroptosis continues to be explored a potential intervention strategy to the treatment of cancers (78,79). Studies have also identified distinct ferroptosis-associated genetic or transcriptome clusters from 1,251 colorectal cancer bulk samples, which are linked with varying biological pathways and clinical outcomes. Ferroptosis-associated patterns linked with the tumor microenvironment diversity and immune response phenotype have been identified (80).

6. Targeting ferroptosis for the treatment of IBD

Lipophilic/radical-trapping antioxidants. This type of therapeutic agent serves as a scavenger for chain-carrying radicals, thus breaking the self-oxidizing chain reaction. The most common chain-breaking radical-trapping or lipophilic antioxidants are aromatic amines and phenols. For the aromatic amines, ferrostatin-1 and liproxstatin-1 have been demonstrated to function as classical ferroptosis inhibitors *in vivo* and *in vitro* (81,82). For example, a previous study demonstrated that the ferroptosis inhibitory activity of ferrostatin-1 and liproxstatin-1 is likely driven by their reactivity as radical-trapping antioxidants rather than their potency as inhibitors of lipoxygenases (81). As previously demonstrated, in a mouse model of DSS-induced IBD, the expression levels of ACSL4 and COX2 were significantly upregulated, while those of GPX4 and FTH1 were downregulated. Treatment with the ferroptosis inhibitors, ferrostatin-1 or liproxstatin-1, mitigated intestinal inflammation by decreasing ACSL4 and COX2, increasing GPX4 and FTH1, and improving mouse body weight and colon length. Moreover, the treatment reduced the expression of Nrf2 and heme oxygenase-1, presenting the suppression of ferroptosis as an effective approach in ameliorating DSS-induced UC in mice (58). Liproxstatin-1 mitigates intestinal barrier injury caused by lipid peroxidation-mediated cell death by inhibiting lipid peroxidation (16).

Ferroptosis has also been demonstrated to participate in the pathophysiology of CD in both human and animal models, indicated by the dysregulation of iron, lipid peroxidation and redox homeostasis. While ACSL4 has been shown to be significantly upregulated and GPX4 to be downregulated in colonic biopsies of patients with CD, other ferroptosis biomarkers, such as MDA, prostaglandin-endoperoxide synthase 2 (PTGS2) and FTH1 are upregulated and GPX4 is downregulated in the colon of the animal model. The administration of ferrostatin-1 in an animal model has been found to alleviate the pathological phenotypes of trinitrobenzene sulfonic acid (TNBS)-induced CD-like colitis (17), providing ferroptosis as a potential therapeutic strategy in treating CD. The role of signal transducer and activator of transcription (STAT)3-mediated ferroptosis, as a ferroptosis-related hub gene, has been reported in *in vitro* and *in vivo* models of colitis, where ferroptosis has been found to increase its expression in DSS-induced colitis, *Salmonella Typhimurium* colitis and in H_2O_2-induced IEC-6 cells. Treatment with ferrostatin-1 reactivates the phosphorylation level of STAT3 (19). The expression of SLC6A14 is significantly elevated and positively correlates with the level of PTGS2 in tissue samples from patients with UC. Moreover, a series of *in vivo* and *in vitro* experiments have indicated that the knockdown of SLC6A14 markedly suppresses ferroptosis. Further analysis has revealed that SLC6A14 inhibits the expression of p21 (RAC1)-activated kinase 6 (PAK6) and the knockdown of PAK6 abolishes the effects of SLC6A14 on RSL3-induced ferroptosis in Caco-2 cells. Mechanistically, SLC6A14 promotes ferroptosis in UC by enhancing the expression of CCAAT enhancer binding protein β (C/EBPβ) and its binding activity to inhibit PAK6 (83), providing the SLC6A14-C/EBPβ-PAK6 axis-mediated ferroptosis a potential therapeutic target for colitis.

α-tocopherol, a natural phenolic substance, is the most active form of vitamin E and serves as an effective suppressor of ferroptosis (84). α-tocopherol specifically breaks both peroxidative chain propagation and inhibits lipoxygenases (85). It has been shown that α-tocopherol and GPX4 cooperatively maintain the lipid redox balance and prevent ferroptosis (84). A previous study demonstrated that the deprivation of vitamin E caused the death of GPX4-deficient mice ~4 weeks after the vitamin E-enriched diet was redrawn (86). These findings indicate that the ferroptosis regulator, GPX4, is crucial for cell survival and proper function, and that α-tocopherol can compensate for the loss of GPX4 by protecting cells against deleterious lipid peroxidation. In a preclinical trial, all 14 patients with IBD (with mild and moderately active UC) responded clinically to d-α-tocopherol therapy, with remission induced in nine of them (64%); of note, no adverse events or hospitalization due to worsened disease activity were reported (87). Although several other studies have reported the ability of α-tocopherol to mitigate experimental IBD by protecting intestinal barrier function, modulating the gut microbiota, accelerating intestinal tissue healing and modulating the immune system (88-90), its inhibition of ferroptosis in IBD requires further research. The anti-ferroptotic potential of other synthetic phenolic compounds, such as 1,8-tetrahydronaphthyridinols has been demonstrated, where the inherent radical-trapping antioxidant reactivity of 1,8-tetrahydronaphthyridinols was similarly effective as ferrostatin-1 and liproxstatin-1 at subverting ferroptosis triggered by either genetic or pharmacological inhibition of the hydroperoxide-detoxifying enzyme GPX4 in mouse fibroblasts and hippocampal cells (81). A schematic summary of the various treatments that target ferroptosis in IBD is presented in Fig. 4.

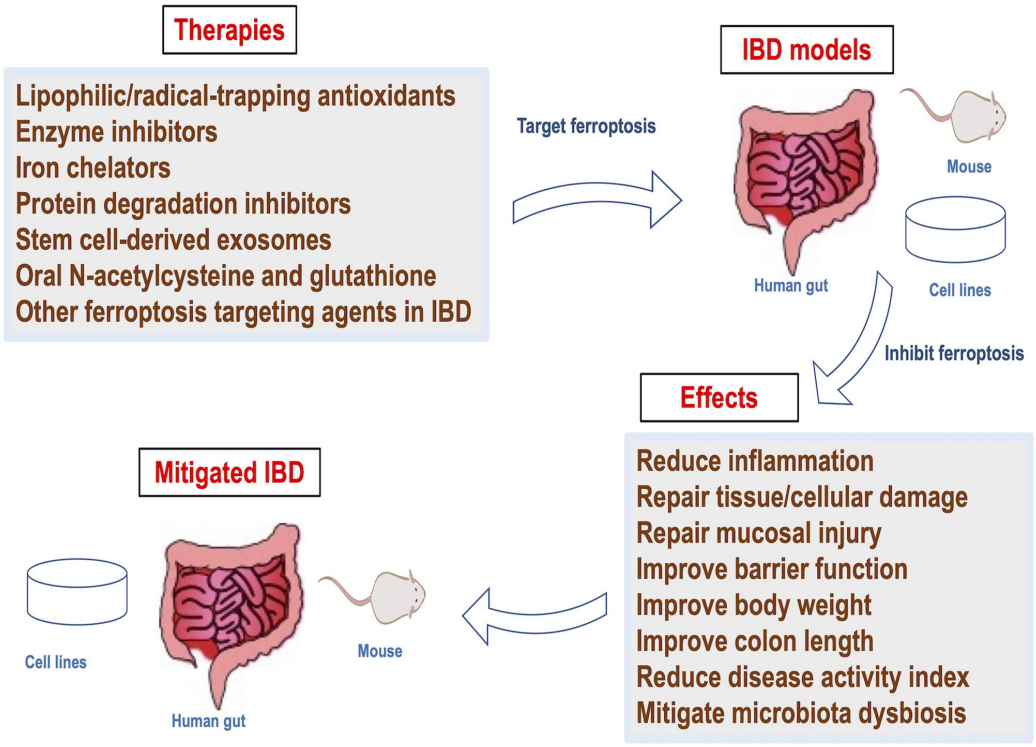

Figure 4. Therapies that target ferroptosis in IBD. As presented in the present review, seven categories of therapies, among others, have been explored for the inhibition of ferroptosis in various IBD models. The majority of these therapies have shown promising effects that lead to the mitigation of IBD. IBD, inflammatory bowel disease.

Iron chelators. Ion is involved in ferroptosis through either the induction of a non-enzymatic iron-mediated Fenton reaction or the activation of iron-containing lipid oxygenases, such as CYP450/cytochrome P450 oxygenases and arachidonate lipoxygenase (ALOX) (38,91). Thus, potent ion chelators, including deferasirox, ciclopirox, deferoxamine and deferiprone, effectively inhibit ferroptosis-induced cell death. The anti-ferroptotic ability of these ion chelators has been largely explored across many conditions, including intestinal cell injury (37), traumatic spinal cord injury (92), osteoarthritis (93), type 2 diabetic osteoporosis (94), atherosclerosis (95), neuroprotection (96) and acute lung injury (97). In assessing the pathogenic role of ROS in UC, Millar *et al* (98) examined the influence of exogenous iron (ferric citrate) and iron chelators (desferrioxamine and 1,10-phenanthroline) on the colonic biopsies of patients with UC and normal control subjects. Through luminol-amplified chemiluminescence, they found that desferrioxamine and phenanthroline decreased chemiluminescence by 47 and 26%, respectively in inactive UC, and by 44 and 42% in active UC. Notably, ferric citrate did not affect the chemiluminescence produced by human colonic mucosa, suggesting that there is sufficient free iron in inflamed biopsies already to drive the Fenton reaction maximally (98). Intestinal epithelial cell injury due to ionizing radiation-induced ferroptosis is effectively abrogated by the iron chelator deferoxamine *in vitro* and *in vivo*. Moreover, the signs of ferroptosis in intestinal epithelial cells, including the upregulation of intracellular iron levels and lipid peroxidation, the increase in PTGS2 mRNA levels, the downregulation of GPX4 mRNA and GSH levels, and significant mitochondrial damage are significantly relieved by deferoxamine treatment (37).

Deferoxamine and deferiprone significantly alleviate the macroscopic and/or pathological features of inflammation in TNBS-induced colitis by reducing colon weight/length ratio, ulcer index and the total colitis index (99). A recent study reported that ferroptosis was induced in mice with UC, as evidenced by ferrous iron accumulation, elevated ROS production, the depletion of superoxide dismutase (SOD) and GSH, and the reduced expression of GPX-4 and ferritin heavy chain (FTH), accompanied by the increased expression of transferrin (TF). However, deferasirox treatment significantly reversed the changes caused by ferroptotic characteristics in DSS-induced mice and reshaped the composition of intestinal microbiota (100). In another study, the combined administration of deferasirox and vitamin D3 mitigated ion induced-injury by decreasing the levels of pro-inflammatory cytokines (IL-1β/IL-6/TNF-α), oxidative stress (MDA/H_2O_2) and apoptotic markers, but increasing IL-10, GSH, SOD1, catalase and GPX4 levels in rats (101). This indicates that deferasirox enhances cellular anti-inflammatory, anti-oxidative stress and iron regulatory pathways in the inhibition of ferroptosis. However, data on ion chelators in IBD therapy are limited.

Enzyme inhibitors. ACSL4, an enzyme that converts fatty acid to fatty acyl-CoA esters, is known to dictate ferroptosis sensitivity by shaping cellular lipid composition (27) and contributes to intestinal tissue injury (68). Moreover, the mitochondrial respiratory chain promotes lipid peroxidation by ALOX or POR (cytochrome P450 reductase); thus, ALOX serves as a critical enzyme of lipid peroxidation that leads to ferroptosis (102). Again, the NOXs (nicotinamide adenine dinucleotide phosphate-oxidase) on the cell membranes are

a resource of cellular ROS for ferroptosis. Therefore, the inhibition of ACSL4, ALOX and NOXs provides promising therapeutic targets for preventing ferroptosis. Reported enzyme inhibitors include ACSL4 inhibitors, such as thiazolidinediones and triacsin C, ALOX inhibitors such as baicalein, cinnamyl-3,4-dihydroxya-cyanocinnamate, PD146176, zileuton, ML351, AA-861 and NCTT-956, and NOX inhibitors, such as GKT137831, diphenylene iodonium and 2-acetylphenothiazine (38,43). For example, the administration of baicalein ameliorates UC by improving the intestinal epithelial barrier (103) and enhances the normalization of the levels of stress response protein and inflammatory cytokine in the intestine, plasma, and marrow of C57BL/6 mice undergoing mitigation of total-body irradiation (104). Zileuton-fed mice develop fewer intestinal polyps and exhibit a significant reduction in systemic and polyp-associated inflammation (105). Furthermore, the NOX inhibitor, diphenyleneiodonium, and the selective NOX1/4 isoform inhibitor, GKT137831, significantly inhibit erastin-induced ROS production, lipid ROS and cell death (106). Data on the exploration of ferroptosis enzyme inhibition in IBD are limited and provide direction for future studies.

Protein degradation inhibitors. As numerous ferroptosis activators possess the capability of inducing GPX4 degradation, which leads to lipid peroxidation, therapeutic agents that block the degradation of GPX4 enhance antioxidant capacity and inhibit ferroptosis. Typical examples of protein degradation inhibitors are dopamine, 2-amino-5-chloro-N,3-dimethylbenzamide (CDDO) and 5-(tetradecyloxy)-2-furoic acid. These inhibitors block FIN56- or erastin-induced GPX4 degradation to prevent ferroptosis (107,108). For instance, dopamine reduces erastin-induced ferrous iron accumulation, GSH depletion and MDA production through the increase in the protein stability of GPX4, a phospholipid hydroperoxidase that protects cells against membrane lipid peroxidation (108). Therefore, a decrease in intestinal dopamine levels is closely related to the development of IBD. Dopamine/dopamine receptor D5 signaling in colonic macrophages controls the development of colitis by modulating M1/M2 macrophage polarization (109) and the activation of the dopamine D2L receptor participates in suppressing colitis-induced weight loss, colon shrinkage, and IL-17 secretion from mesenteric lymph node lymphocytes in response to CD3/CD28 stimulation in mice (110). Therefore, dopamine serves not only as an anti-ferroptotic agent, but also as a potent immune response regulator to ameliorate colitis. In other research, CDDO, a compound known to inhibit heat shock protein 90 (HSP90), was found to potently inhibit both ferroptosis and necroptosis (111). Erastin-activated ferroptosis was found to increase the levels of lysosome-associated membrane protein 2a, also known as CD107b, to promote chaperone-mediated autophagy (CMA), which in turn, promotes the degradation of GPX4. Notably, the inhibition of CMA stabilized GPX4 and reduced ferroptosis (111). These findings suggest that the use of agents that inhibit GPX4 degradation may provide an effective means of mitigating ferroptosis-related lethal effects in IBD.

Stem cell-derived exosomes. Stem cells and their derived exosomes have been largely explored as promising therapeutic agents in the treatment of human IBD (112-114) and experimental IBD (115-117). As regards the the targetted treatment of IBD by inhibiting ferroptosis, a recent study confirmed that relative to healthy individuals, patients with UC exhibit higher levels of iron, ACSL4 and MDA, whereas they exhibit decreased levels of GSH and GPX in colon tissues (118). In that study, in the animal model of IBD, exosomes derived from endometrial regenerative cells attenuated the clinical symptoms of colitis and tissue damage by enhancing the expression of GSH and GPX4, but reducing the levels of iron, MDA and ACSL4 in the colons of mice with colitis. Moreover, *in vitro* analysis revealed that the exosomes rescued erastin-induced cell death and upregulated the levels of GSH and GPX4, while downregulating the levels of iron, MDA and ACSL4 (118). This illustrates the involvement of ferroptosis in the pathogenesis of IBD and the potential of stem cell-derived exosomes to mitigate colitis through the downregulation of intestinal ferroptosis.

In other studies, mesenchymal stem cell-derived exosomes (MSC-Ex) were protected against ferroptosis via the exosomal-mediated stabilization of SLC7A11 in acute liver injury. It was demonstrated that the MSC-Ex triggered the increased expression of SLC7A11 protein, CD44 and OTUB1, and the activation of system X_C^- to prevent ferroptosis (119). The stability of SLC7A11 and the consequent activation of system X_C^- may partly be attributed to CD44 expression, which suppresses ferroptosis in an OTUB1-dependent manner (120). Moreover, MSC-Ex have been found to inhibit the production of ROS and ferrous iron, upregulate the expression of ferroptosis suppressor FSP1, and enhance the repair of neurological function in mice. Mechanistically, MSC-Ex lncGm36569 prevents ferroptosis-induced neuronal cell dysfunction through the miR-5627-5p/FSP1 axis (121), and MSC-Ex also attenuate renal ischemia/reperfusion injury by interacting with SRSF1 to regulate ASCL4-mediated ferroptosis (122), attenuate myocardial injury by inhibiting ferroptosis in acute myocardial infarction mice (123), and inhibit the ferroptosis of hepatic stellate cells by regulating the xCT/GPX4 axis (124). These observations indicate the promising therapeutic outcomes of the MSC-Ex-induced inhibition of ferroptosis in cell protection. This may provide future direction for research in IBD therapy exploration.

Oral N-acetylcysteine and glutathione. Replenishing cysteine/glutathione deficiency is also an effective method with which to abrogate oxidative stress and its associated lipoperoxidation that characterizes ferroptosis. Thus, the administration of *N*-acetylcysteine, a cysteine prodrug, replenishes intracellular GSH levels and serves as a safe and well-tolerated antidote for cysteine/GSH deficiency (125). In addition to *N*-acetylcysteine, oral GSH and sublingual form of GSH also serves as potent GSH supplementation in humans (126). As previously demonstrated, the *in vivo* administration of *N*-acetylcysteine attenuates acute TNBS-induced colitis in rats through increased mucosal GSH levels, reducing the extent of intestinal mucosal injury and TNBS-induced ROS overgeneration (127). However, further studies are required to further explore treatments that directly replenish cysteine/glutathione stores as a potential therapy for IBD and other ferroptosis-induced intestinal injuries.

Other agents targeting ferroptosis in IBD. Curculigoside has been shown to alleviate colitis in mice by reversing ferroptosis-related changes, such as iron overload, GSH depletion, excessive ROS and MDA production, and the decreased expression of SOD and GPX4. In IEC-6 cells, curculigoside enhances selenium sensitivity and promotes GPX4 transcription. The selective knockdown of GPX4 significantly inhibits the protective effects of curculigoside on cell death and prevents its downregulation of GSH, MDA, and lactate dehydrogenase activity in ferroptotic IEC-6 (18). The protease furin, a proteolytic enzyme found to exert protective effects in several autoimmune diseases, inhibits DSS-induced ferroptosis-like injury in colon epithelial cells *in vivo* and *in vitro* by upregulating GPX4. Mechanistically, furin alleviated the cell injury by activating the Nrf2-GPX4 signaling pathway in mice and NCM460 cells (128). In a recent study, zero-valence selenium-enriched Prussian blue nanozymes (Se-HMPB nanozymes), multifunctional nanozymes capable of scavenging ROS and inhibiting ferroptosis or T-cell differentiation, were explored for IBD therapy (129). The researchers reported that Se-HMPB nanozymes effectively scavenged various ROS in inflammatory tissues and enhanced GPX activity, inhibiting ferroptosis and reversing the lipid peroxidation of intestinal epithelial cells to preserve intestinal barrier integrity in UC. Additionally, the construct inhibited T-cell differentiation in a CD model, regulating the intestinal immune barrier (129), and thus presenting great potential for IBD therapy.

Dipeptidyl peptidase-4 (DPP4) has been implicated in the pathogenesis of IBD and as a potential biomarker (130). It has been shown that TP53 limits erastin-induced ferroptosis by blocking DPP4 activity in a transcription-independent manner. The loss of TP53 prevents the nuclear accumulation of DPP4, and thus facilitates plasma-membrane-associated DPP4-dependent lipid peroxidation, which finally results in ferroptosis (131). In experimental IBD, the DPP4 inhibitor, linagliptin, mitigates colitis by inhibiting colonic DPP4 activity, suppressing colonic IL-6, TNF-α, and myeloperoxidase, and the upregulation of IL-10. Linagliptin also reduces intestinal mucosal oxidative stress by inhibiting lipid peroxides and augmenting GSH, GPX and total antioxidant capacity (132). In similar studies, linagliptin has exhibited promising anti-inflammatory activity against acetic acid-induced colitis through the stimulation of the AMPK/SIRT1/PGC-1α pathway, as well as the inhibition of the JAK2/STAT3 signaling pathway, which may partly be mediated through AMPK activation (133).

7. Targeting ferroptosis in other intestinal diseases

Intestinal I/R injury. Similar to other gastrointestinal diseases, intestinal I/R injury is associated with multiple types of regulated cell death, including autophagy, apoptosis and necroptosis, and the discovery of ferroptosis has triggered the exploration of potential mechanisms of ferroptotic cell death in intestinal I/R injury. ROS generation and lipid peroxidation are associated with intestinal I/R injury and are primary contributors to the initiation and execution of ferroptosis (134). A previous study found that the inhibition of ACSL4, the key enzyme that regulates lipid composition, prior to intestinal reperfusion, protected against ferroptosis and cell death (68). The authors of that study concluded that ferroptosis was closely associated with intestinal I/R injury, and ACSL4 participated in the lethal process, while specificityprotein 1 served as a crucial transcription factor for the increased expression of ACSL4 (68). Depleted GSH levels and a reduced SOD activity, as well as elevated MDA levels, have been observed in animal models of intestinal tissue I/R injury (135,136). These observations implicate the participation of ferroptosis in I/R-induced intestinal injury; however, studies on the underlying mechanisms are largely lacking.

Necrotizing enterocolitis. Necrotizing enterocolitis is the most common gastrointestinal disease among newborns and research has established the involvement of several types of regulated cell death in the gut barrier dysfunction that characterizes the disease (137,138). In a previous study, through bioinformatics analyses, ferroptosis was identified to play a role in necrotizing enterocolitis, where *ACSL4* gene expression levels were significantly upregulated and positively correlated with ferroptosis, and the influx of multiple immune cells, including macrophages, neutrophils, activated dendritic cells and regulatory T-cells. The inhibition of ferroptosis significantly attenuated necrotizing enterocolitis in newborn mice (69). In other studies, the inhibition of *ACSL4* has been proven to be a viable therapeutic approach to preventing ferroptosis-related inflammation, tissue injury, and even tumors (139,140), as ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition (27). Moreover, ROS, a product of ferroptosis, has been found to accumulate in the damaged gut tissues of infants with necrotizing enterocolitis and to serve as a regulatory point (ROS-NF-κB axis) in ameliorating inflammation in necrotizing enterocolitis (141).

CAC. The inhibition of ferroptosis as a treatment approach for CAC presents varying outcomes and warrants further investigations. On the one hand, the inhibition of ferroptosis in intestinal tissues by OTSSP167, a MELK inhibitor, alleviates colitis and abrogates the occurrence and progression of CAC by reducing M1 macrophage polarization, macrophage infiltration, downregulating the secretion of pro-inflammatory factors, and significantly repairing intestinal damage. Mechanistically, the treatment inhibits the AKT/IKK/p65 and ERK/IKK/p65 signaling cascades *in vivo* and *in vitro* to mitigate colitis and its related colorectal cancer (142). On the other hand, the administration of the ferroptosis inhibitor, ferrostatin-1, results in an increased incidence of CAC. This effect is exacerbated in mice fed a high-fat diet, as they exhibit an increased tumor number and a higher degree of dysplasia following the repression of lipid peroxidation and ferroptosis marker expression in mouse colon tissue (70), providing a new perspective for the prevention of CAC. Additional studies are required in this field to provide clarity and explore the underlying mechanisms. The therapeutic agents, key effects and mechanisms of ferroptosis targets in IBD and other intestinal diseases are summarized in Table I.

8. Conclusions and future perspectives

Ferroptosis is driven by iron-dependent phospholipid peroxidation and is modulated by several cellular metabolic activities,

Table I. Therapeutic targets of ferroptosis in IBD and other gastrointestinal diseases.

Therapeutic agent	Disease/model	Mechanism	Key effects	(Refs.)
Ferrostatin-1, liproxstatin-1, or deferiprone	DSS-induced UC in mice	Ferroptosis inhibition via the Nrf2/HO-1 signaling pathway	Decreased COX2 and ACSL4 but increased GPX4 and FTH1 Reduced inflammation indexes and malanoyl dialdehyde levels	(58)
Ferrostatin-1	TNBS-induced CD-like colitis in mice	Inhibition of ferroptosis	Ameliorates the pathological phenotypes of TNBS-induced colitis Reduced PTGS2, MDA and FTH1, and increased GPX4 levels	(17)
Ferrostatin-1	DSS-induced colitis, *Salmonella Typhimurium* colitis, and H_2O_2-induced IEC-6 cells	Ferroptosis is closely associated with the development of colitis via the *STAT3* gene	Reactivates the phosphorylation level of STAT3	(19)
Desferrioxamine and phenanthroline	Colonic mucosa biopsies from patients with UC	Reduction of chemiluminescence generated by reactive oxygen species	Decreased mucosal reactive oxygen species production in both active and inactive UC	(98)
N-acetylcysteine	TNBS + ethanol-induced colitis in rats	Increase of mucosal GSH levels to mitigate acute colitis	Increased GSH stores 2-fold, decreased extent of mucosal injury	(127)
Curculigoside	DSS-induced colitis and IEC-6 cells	Ferroptosis inhibition via the induction of GPX4	Decreased ROS, MDA, and ion but increased GSH, SOD and GPX4 Relieved colitis	(18)
Linagliptin	TNBS-induced colitis in rats	Inhibits the IL-6/JAK2/STAT3 pathway via downregulating p-JAK2/JAK2 and p-STAT3/STAT3 proteins	Suppresses colonic IL-6, TNF-α, and myeloperoxidase and upregulates IL-10	(132)
		Inhibits the HMGB1/RAGE/NF-κB cascade via lowering HMGB1, RAGE, and p-NF-κB p65/NF-κB p65 proteins	Inhibits mucosal oxidative stress by reducing lipid peroxides and augmenting GSH, GPX, and total antioxidant capacity	
Endometrial regenerative cells-derived exosome	DSS-induced colitis and erastin-treated NCM460 human intestinal epithelial cell line	Inhibition of ferroptosis	Increased GSH and GPX4 but reduced iron, MDA, and ACSL4 in the colon	(118)
Furin	DSS-induced colitis in mice and DSS-treated NCM460 cells	Inhibits ferroptosis-like injury by activating the Nrf2-Gpx4 signaling pathway	Upregulated GPX4 and prevented cell/tissue injury *in vivo* and *in vitro*. Alleviates DSS-induced damage to mitochondrial membranes	(128)
OTSSP167	DSS-induced colitis and CAC	Inhibits ferroptosis via the AKT/IKK/p65 and ERK/IKK/p65 signaling cascades *in vivo* and *in vitro*	Alleviation of intestinal inflammation and the occurrence of CAC	(142)
Ferrostatin-1 (ferroptosis inhibitor)	AOM/DSS-induced mouse CAC	CAC aggravates through the evasion of ferroptosis in the ER stress-mediated pathway	Ferrostatin-1 treatment increases the incidence of CAC. The induction of ferroptosis partly abolished the pro-tumor effects of a high-fat diet on CAC	(70)

Table I. Continued.

Therapeutic agent	Disease/model	Mechanism	Key effects	(Refs.)
Liproxstatin-1	I/R-induced intestinal injury	Inhibit ferroptosis by reducing ACSL4 activation and function	Increases GPX4 and reduces COX2, lipid peroxidation, serum LDH, TNF-α, and IL-6 levels, and alleviates intestinal I/R injury	(68)
Resveratrol, dioscin	I/R-induced intestinal injury	Inhibition of oxidative stress	Reduces the levels of MDA, MPO, and NO, but increase SOD activity, thus, ameliorating intestinal I/R injury	(134, 135)
ACSL4 knockdown	Necrotizing enterocolitis	ACSL4-mediated ferroptosis	ACSL4 expression is positively associated with ferroptosis, inflammation, and the abundance of macrophage, activated dendritic cells, neutrophils, and regulatory T-cells	(69)
Antioxidant peptide from tuna backbone protein (APTBP)	Necrotizing enterocolitis	ROS-NF-κB-mediated inflammation	Reduces intracellular ROS and inflammatory cytokines	(141)

IBD, inflammatory bowel disease; DSS, dextran sulfate sodium; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase 1; TNBS, trinitrobenzene sulfonic acid; CD, Chrohn's disease; COX2, cyclooxygenase 2; ACSL4, acyl-CoA synthetase long-chain family member 4; GPX4, glutathione peroxidase 4; FTH1, ferritin heavy chain 1; PTGS2, prostaglandin-endoperoxide synthase 2; MDA, malondialdehyde; STAT3, signal transducer and activator of transcription 3; UC, ulcerative colitis; GSH, glutathione; ROS, reactive oxygen species; SOD, superoxide dismutase; HMGB1, high mobility group box 1; RAGE, receptor for advanced glycosylation end product; CAC, colitis-associated colorectal cancer; I/R, ischemia/reperfusion; LDH, lactate dehydrogenase; AOM, azoxymethane.

such as redox homeostasis, iron transport and metabolism, mitochondrial activity, and the metabolism of lipids, amino acids and sugars, as well as multiple signaling pathways relevant to disease processes. The regulation of key mediators, including GPX4, ACSL4, SLC7A11 and p53 is crucial in modulating ferroptosis-associated intestinal diseases. Although the pathophysiological role of ferroptosis continues to be explored, its involvement in several human diseases, including IBD has been documented. A number of studies, as those aforementioned, have demonstrated the participation of ferroptosis in IBD and the ability of several therapeutic agents to mitigate the lethal effect associated with ferroptosis in IBD. Notably, the therapeutic regulation of ferroptosis has exhibited promising outcomes as a novel treatment avenue for IBD and other intestinal diseases. However, the field is still relatively new and requires further studies and the extensive exploration of related mechanisms. For example, although the uncontrolled peroxidation of PUFA-PLs has been identified as the most downstream step in the process of ferroptosis, the exact mechanisms through which cells ultimately die warrant further elucidation. Moreover, the discovery of precise biomarkers for *in vivo* ferroptosis may enhance the current understanding of the pathophysiological role of this cell death modality. This will also provide more precise and effective targets for the inhibition of ferroptosis. Again, the current experimental practice of preventing cell death by a single ferroptosis inhibitor may not be sufficient evidence that ferroptosis participates in a given process, since the association between ferroptotic cell death and iron/lipid peroxides remains largely unclear. Further evidence is required in order to better understand these links, as well as to provide transcriptional regulators and signaling pathways associated with ferroptosis in IBD development and progression.

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Availability of data and materials

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Authors' contributions

DKWO and JY were involved in the conception and design of the study, as well as in the collection and/or assembly, analysis and interpretation of data to be included in the review, and in the writing of the manuscript. ZW was involved in the provision of study materials, as well as in the interpretation and analysis of data to be included in the review. FM and ZZ were involved in the design of the study, as well as in the interpretation and analysis of data to be included in the review, and in the writing of the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Hodson R: Inflammatory bowel disease. Nature 540: S97, 2016.
2. Cai Z, Wang S and Li J: Treatment of inflammatory bowel disease: A comprehensive review. Front Med 8: 765474, 2021.
3. Na SY and Moon W: Perspectives on current and novel treatments for inflammatory bowel disease. Gut Liver 13: 604-616, 2019.
4. Ocansey DKW, Xu X, Zhang L and Mao F: Mesenchymal stem cell-derived exosome: The likely game-changer in stem cell research. BIOCELL 46: 1169-1172, 2022.
5. Xu M, Tao J, Yang Y, Tan S, Liu H, Jiang J, Zheng F and Wu B: Ferroptosis involves in intestinal epithelial cell death in ulcerative colitis. Cell Death Dis 11: 86, 2020.
6. Xu S, He Y, Lin L, Chen P, Chen M and Zhang S: The emerging role of ferroptosis in intestinal disease. Cell Death Dis 12: 289, 2021.
7. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, *et al*: Ferroptosis: An iron-dependent form of nonapoptotic cell death. Cell 149: 1060-1072, 2012.
8. Otasevic V, Vucetic M, Grigorov I, Martinovic V and Stancic A: Ferroptosis in different pathological contexts seen through the eyes of mitochondria. Oxid Med Cell Longev 2021: 5537330, 2021.
9. Battaglia AM, Chirillo R, Aversa I, Sacco A, Costanzo F and Biamonte F: Ferroptosis and cancer: Mitochondria meet the 'iron maiden' cell death. Cells 9: 1505, 2020.
10. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, Kang R and Tang D: Ferroptosis: Process and function. Cell Death Differ 23: 369-379, 2016.
11. Xu C, Liu Z and Xiao J: Ferroptosis: A double-edged sword in gastrointestinal disease. Int J Mol Sci 22: 12403, 2021.
12. Gao W, Zhang T and Wu H: Emerging pathological engagement of ferroptosis in gut diseases. Oxid Med Cell Longev 2021: 4246255, 2021.
13. Dixon SJ and Stockwell BR: The hallmarks of ferroptosis. Annu Rev Cancer Biol 3: 35-54, 2019.
14. Sui X, Zhang R, Liu S, Duan T, Zhai L, Zhang M, Han X, Xiang Y, Huang X, Lin H and Xie T: RSL3 drives ferroptosis through GPX4 inactivation and ROS production in colorectal cancer. Front Pharmacol 9: 1371, 2018.
15. Huang J, Zhang J, Ma J, Ma J, Liu J, Wang F and Tang X: Inhibiting ferroptosis: A novel approach for ulcerative colitis therapeutics. Oxid Med Cell Longev 2022: 9678625, 2022.
16. Ma D, Jiang P, Jiang Y, Li H and Zhang D: Effects of lipid peroxidation-mediated ferroptosis on severe acute pancreatitis-induced intestinal barrier injury and bacterial translocation. Oxid Med Cell Longev 2021: 6644576, 2021.
17. Xu J, Liu S, Cui Z, Wang X, Ning T, Wang T, Zhang N, Xie S, Min L, Zhang S, *et al*: Ferrostatin-1 alleviated TNBS induced colitis via the inhibition of ferroptosis. Biochem Biophys Res Commun 573: 48-54, 2021.
18. Wang S, Liu W, Wang J and Bai X: Curculigoside inhibits ferroptosis in ulcerative colitis through the induction of GPX4. Life Sci 259: 118356, 2020.
19. Huang F, Zhang S, Li X, Huang Y, He S and Luo L: STAT3-mediated ferroptosis is involved in ulcerative colitis. Free Radic Biol Med 188: 375-385, 2022.

20. Cao JY and Dixon SJ: Mechanisms of ferroptosis. Cell Mol Life Sci 73: 2195-2209, 2016.
21. Tang D, Chen X, Kang R and Kroemer G: Ferroptosis: Molecular mechanisms and health implications. Cell Res 31: 107-125, 2021.
22. Jiang X, Stockwell BR and Conrad M: Ferroptosis: Mechanisms, biology and role in disease. Nat Rev Mol Cell Biol 22: 266-282, 2021.
23. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, *et al*: Regulation of ferroptotic cancer cell death by GPX4. Cell 156: 317-331, 2014.
24. Hu W, Liang K, Zhu H, Zhao C, Hu H and Yin S: Ferroptosis and its role in chronic diseases. Cells 11: 2040, 2022.
25. Conrad M and Pratt DA: The chemical basis of ferroptosis. Nat Chem Biol 15: 1137-1147, 2019.
26. Dixon SJ, Winter GE, Musavi LS, Lee ED, Snijder B, Rebsamen M, Superti-Furga G and Stockwell BR: Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. ACS Chem Biol 10: 1604-1609, 2015.
27. Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, *et al*: ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. Nat Chem Biol 13: 91-98, 2017.
28. Bano I, Horky P, Abbas SQ, Majid M, Bilal AHM, Ali F, Behl T, Hassan SSU and Bungau S: Ferroptosis: A new road towards cancer management. Molecules 27: 2129, 2022.
29. Ursini F, Maiorino M and Gregolin C: The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim Biophys Acta 839: 62-70, 1985.
30. Skouta R, Dixon SJ, Wang J, Dunn DE, Orman M, Shimada K, Rosenberg PA, Lo DC, Weinberg JM, Linkermann A and Stockwell BR: Ferrostatins inhibit oxidative lipid damage and cell death in diverse disease models. J Am Chem Soc 136: 4551-4556, 2014.
31. Chen X, Yu C, Kang R and Tang D: Iron metabolism in ferroptosis. Front Cell Dev Biol 8: 590226, 2020.
32. Hou W, Xie Y, Song X, Sun X, Lotze MT, Zeh HJ III, Kang R and Tang D: Autophagy promotes ferroptosis by degradation of ferritin. Autophagy 12: 1425-1428, 2016.
33. Gao M, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB and Jiang X: Role of mitochondria in ferroptosis. Mol Cell 73: 354-363.e3, 2019.
34. Gao M, Monian P, Pan Q, Zhang W, Xiang J and Jiang X: Ferroptosis is an autophagic cell death process. Cell Res 26: 1021-1032, 2016.
35. Chen X, Kang R, Kroemer G and Tang D: Organelle-specific regulation of ferroptosis. Cell Death Differ 28: 2843-2856, 2021.
36. Lee H, Zandkarimi F, Zhang Y, Meena JK, Kim J, Zhuang L, Tyagi S, Ma L, Westbrook TF, Steinberg GR, *et al*: Energy-stress-mediated AMPK activation inhibits ferroptosis. Nat Cell Biol 22: 225-234, 2020.
37. Zhou H, Zhou YL, Mao JA, Tang LF, Xu J, Wang ZX, He Y and Li M: NCOA4-mediated ferritinophagy is involved in ionizing radiation-induced ferroptosis of intestinal epithelial cells. Redox Biol 55: 102413, 2022 (Epub ahead of print).
38. Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS and Stockwell BR: Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. Proc Natl Acad Sci USA 113: E4966-E4975, 2016.
39. Kobayashi Y, Ohfuji S, Kondo K, Fukushima W, Sasaki S, Kamata N, Yamagami H, Fujiwara Y, Suzuki Y and Hirota Y; Japanese Case-Control Study Group for Ulcerative Colitis: Association of dietary fatty acid intake with the development of ulcerative colitis: A multicenter case-control study in Japan. Inflamm Bowel Dis 27: 617-628, 2021.
40. John S, Luben R, Shrestha SS, Welch A, Khaw KT and Hart AR: Dietary n-3 polyunsaturated fatty acids and the aetiology of ulcerative colitis: A UK prospective cohort study. Eur J Gastroenterol Hepatol 22: 602-606, 2010.
41. Lee JY, Nam M, Son HY, Hyun K, Jang SY, Kim JW, Kim MW, Jung Y, Jang E, Yoon SJ, *et al*: Polyunsaturated fatty acid biosynthesis pathway determines ferroptosis sensitivity in gastric cancer. Proc Natl Acad Sci USA 117: 32433-32442, 2020.
42. Ursini F and Maiorino M: Lipid peroxidation and ferroptosis: The role of GSH and GPx4. Free Radic Biol Med 152: 175-185, 2020.
43. Chen X, Li J, Kang R, Klionsky DJ and Tang D: Ferroptosis: Machinery and regulation. Autophagy 17: 2054-2081, 2021.
44. Conrad M and Sato H: The oxidative stress-inducible cystine/glutamate antiporter, system x (c) (-): Cystine supplier and beyond. Amino Acids 42: 231-246, 2012.
45. Ahmed I, Manno FAM, Manno SHC, Liu Y, Zhang Y and Lau C: Detection of lithium in breast milk and in situ elemental analysis of the mammary gland. Biomed Opt Express 9: 4184-4195, 2018.
46. Ingold I, Berndt C, Schmitt S, Doll S, Poschmann G, Buday K, Roveri A, Peng X, Porto Freitas F, Seibt T, *et al*: Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. Cell 172: 409-422.e21, 2018.
47. Li S, He Y, Chen K, Sun J, Zhang L, He Y, Yu H and Li Q: RSL3 Drives ferroptosis through NF-κB pathway activation and GPX4 depletion in glioblastoma. Oxid Med Cell Longev 2021: 2915019, 2021.
48. Zhang DL, Ghosh MC and Rouault TA: The physiological functions of iron regulatory proteins in iron homeostasis-an update. Front Pharmacol 5: 124, 2014.
49. Yang L, Cao LM, Zhang XJ and Chu B: Targeting ferroptosis as a vulnerability in pulmonary diseases. Cell Death Dis 13: 649, 2022.
50. Brown CW, Amante JJ, Chhoy P, Elaimy AL, Liu H, Zhu LJ, Baer CE, Dixon SJ and Mercurio AM: Prominin2 drives ferroptosis resistance by stimulating iron export. Dev Cell 51: 575-586.e4, 2019.
51. Kobayashi Y, Ohfuji S, Kondo K, Fukushima W, Sasaki S, Kamata N, Yamagami H, Fujiwara Y, Suzuki Y and Hirota Y; Japanese Case-Control Study Group for Ulcerative Colitis: Association between dietary iron and zinc intake and development of ulcerative colitis: A case-control study in Japan. J Gastroenterol Hepatol 34: 1703-1710, 2019.
52. Sivaprakasam S, Ristic B, Mudaliar N, Hamood AN, Colmer-Hamood J, Wachtel MS, Nevels AG, Kottapalli KR and Ganapathy V: Hereditary hemochromatosis promotes colitis and colon cancer and causes bacterial dysbiosis in mice. Biochem J 477: 3867-3883, 2020.
53. Wenz C, Faust D, Linz B, Turmann C, Nikolova T, Bertin J, Gough P, Wipf P, Schröder AS, Krautwald S and Dietrich C: t-BuOOH induces ferroptosis in human and murine cell lines. Arch Toxicol 92: 759-775, 2018.
54. Wenz C, Faust D, Linz B, Turmann C, Nikolova T and Dietrich C: Cell-cell contacts protect against t-BuOOH-induced cellular damage and ferroptosis in vitro. Arch Toxicol 93: 1265-1279, 2019.
55. Chen X, Kang R, Kroemer G and Tang D: Broadening horizons: The role of ferroptosis in cancer. Nat Rev Clin Oncol 18: 280-296, 2021.
56. Chen GQ, Benthani FA, Wu J, Liang D, Bian ZX and Jiang X: Artemisinin compounds sensitize cancer cells to ferroptosis by regulating iron homeostasis. Cell Death Differ 27: 242-254, 2020.
57. Gaschler MM, Andia AA, Liu H, Csuka JM, Hurlocker B, Vaiana CA, Heindel DW, Zuckerman DS, Bos PH, Reznik E, *et al*: $FINO_2$ initiates ferroptosis through GPX4 inactivation and iron oxidation. Nat Chem Biol 14: 507-515, 2018.
58. Chen Y, Zhang P, Chen W and Chen G: Ferroptosis mediated DSS-induced ulcerative colitis associated with Nrf2/HO-1 signaling pathway. Immunol Lett 225: 9-15, 2020.
59. Hu Y, Sun S and Li H: Identification and functional exploration of Ferroptosis and Immune Related Long Non-Coding RNA in Inflammatory bowel disease. Res Sq, 2022.
60. Patankar JV and Becker C: Cell death in the gut epithelium and implications for chronic inflammation. Nat Rev Gastroenterol Hepatol 17: 543-556, 2020.
61. Luo L, Zhang S, Guo N, Li H and He S: ACSF2-mediated ferroptosis is involved in ulcerative colitis. Life Sci 313: 121272, 2023.
62. Cui DJ, Chen C, Yuan WQ, Yang YH and Han L: Integrative analysis of ferroptosis-related genes in ulcerative colitis. J Int Med Res 49: 3000605211042975, 2021.
63. Ma H, Shu Q, Li D, Wang T, Li L, Song X, Lou K and Xu H: Accumulation of intracellular ferrous iron in inflammatory-activated macrophages. Biol Trace Elem Res 201: 2303-2310, 2023.
64. Maloy KJ and Powrie F: Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature 474: 298-306, 2011.
65. Panda SK, Peng V, Sudan R, Ulezko Antonova A, Di Luccia B, Ohara TE, Fachi JL, Grajales-Reyes GE, Jaeger N, Trsan T, *et al*: Repression of the aryl-hydrocarbon receptor prevents oxidative stress and ferroptosis of intestinal intraepithelial lymphocytes. Immunity 56: 797-812.e4, 2023.

66. Wang L, Han R, Zang K, Yuan P and Qin H: Deficiency in glutathione synthesis and reduction contributes to the pathogenesis of colitis-related liver injury. Zhong Nan Da Xue Xue Bao Yi Xue Ban 47: 271-279, 2022 (In English, Chinese).
67. Lin JH, Walter P and Yen TSB: Endoplasmic reticulum stress in disease pathogenesis. Annu Rev Pathol 3: 399-425, 2008.
68. Li Y, Feng D, Wang Z, Zhao Y, Sun R, Tian D, Liu D, Zhang F, Ning S, Yao J and Tian X: Ischemia-induced ACSL4 activation contributes to ferroptosis-mediated tissue injury in intestinal ischemia/reperfusion. Cell Death Differ 26: 2284-2299, 2019.
69. Dang D, Zhang C, Meng Z, Lv X, Li Z, Wei J and Wu H: Integrative analysis links ferroptosis to necrotizing enterocolitis and reveals the role of ACSL4 in immune disorders. iScience 25: 105406, 2022.
70. Zhang X, Li W, Ma Y, Zhao X, He L, Sun P and Wang H: High-fat diet aggravates colitis-associated carcinogenesis by evading ferroptosis in the ER stress-mediated pathway. Free Radic Biol Med 177: 156-166, 2021.
71. Chen J, Li X, Ge C, Min J and Wang F: The multifaceted role of ferroptosis in liver disease. Cell Death Differ 29: 467-480, 2022.
72. Zhang X, Du L, Qiao Y, Zhang X, Zheng W, Wu Q, Chen Y, Zhu G, Liu Y, Bian Z, *et al*: Ferroptosis is governed by differential regulation of transcription in liver cancer. Redox Biol 24: 101211, 2019.
73. Wang Y, Zhang Z, Sun W, Zhang J, Xu Q, Zhou X and Mao L: Ferroptosis in colorectal cancer: Potential mechanisms and effective therapeutic targets. Biomed Pharmacother 153: 113524, 2022.
74. Ouyang S, Li H, Lou L, Huang Q, Zhang Z, Mo J, Li M, Lu J, Zhu K, Chu Y, *et al*: Inhibition of STAT3-ferroptosis negative regulatory axis suppresses tumor growth and alleviates chemoresistance in gastric cancer. Redox Biol 52: 102317, 2022.
75. Xu T, Ding W, Ji X, Ao X, Liu Y, Yu W and Wang J: Molecular mechanisms of ferroptosis and its role in cancer therapy. J Cell Mol Med 23: 4900-4912, 2019.
76. Wang R, Su Q, Yin H, Wu D, Lv C and Yan Z: Inhibition of SRSF9 enhances the sensitivity of colorectal cancer to erastin-induced ferroptosis by reducing glutathione peroxidase 4 expression. Int J Biochem Cell Biol 134: 105948, 2021.
77. Zhang L, Liu W, Liu F, Wang Q, Song M, Yu Q, Tang K, Teng T, Wu D, Wang X, *et al*: Corrigendum to 'IMCA induces ferroptosis mediated by SLC7A11 through the AMPK/mTOR pathway in colorectal cancer'. Oxid Med Cell Longev 2020: 6901472, 2020.
78. Xia Y, Liu S, Li C, Ai Z, Shen W, Ren W and Yang X: Discovery of a novel ferroptosis inducer-talaroconvolutin A-killing colorectal cancer cells in vitro and in vivo. Cell Death Dis 11: 988, 2020.
79. Gao W, Huang Z, Duan J, Nice EC, Lin J and Huang C: Elesclomol induces copper-dependent ferroptosis in colorectal cancer cells via degradation of ATP7A. Mol Oncol 15: 3527-3544, 2021.
80. Luo W, Dai W, Li Q, Mo S, Han L, Xiao X, Gu R, Xiang W, Ye L, Wang R, *et al*: Ferroptosis-associated molecular classification characterized by distinct tumor microenvironment profiles in colorectal cancer. Int J Biol Sci 18: 1773-1794, 2022.
81. Zilka O, Shah R, Li B, Friedmann Angeli JP, Griesser M, Conrad M and Pratt DA: On the mechanism of cytoprotection by ferrostatin-1 and liproxstatin-1 and the role of lipid peroxidation in ferroptotic cell death. ACS Cent Sci 3: 232-243, 2017.
82. Kajarabille N and Latunde-Dada GO: Programmed cell-death by ferroptosis: Antioxidants as mitigators. Int J Mol Sci 20: 4968, 2019.
83. Chen Y, Yan W, Chen Y, Zhu J, Wang J, Jin H, Wu H, Zhang G, Zhan S, Xi Q, *et al*: SLC6A14 facilitates epithelial cell ferroptosis via the C/EBPβ-PAK6 axis in ulcerative colitis. Cell Mol Life Sci 79: 563, 2022.
84. Hu Q, Zhang Y, Lou H, Ou Z, Liu J, Duan W, Wang H, Ge Y, Min J, Wang F and Ju Z: GPX4 and vitamin E cooperatively protect hematopoietic stem and progenitor cells from lipid peroxidation and ferroptosis. Cell Death Dis 12: 706, 2021.
85. Maiorino M, Conrad M and Ursini F: GPx4, lipid peroxidation, and cell death: Discoveries, rediscoveries, and open issues. Antioxid Redox Signal 29: 61-74, 2018.
86. Carlson BA, Tobe R, Yefremova E, Tsuji PA, Hoffmann VJ, Schweizer U, Gladyshev VN, Hatfield DL and Conrad M: Glutathione peroxidase 4 and vitamin E cooperatively prevent hepatocellular degeneration. Redox Biol 9: 22-31, 2016.
87. Mirbagheri SA, Nezami BG, Assa S and Hajimahmoodi M: Rectal administration of d-alpha tocopherol for active ulcerative colitis: A preliminary report. World J Gastroenterol 14: 5990-5995, 2008.
88. Rachmawati H, Pradana AT, Safitri D and Adnyana IK: Multiple functions of D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) as curcumin nanoparticle stabilizer: In vivo kinetic profile and anti-ulcerative colitis analysis in animal model. Pharmaceutics 9: 24, 2017.
89. Liu KY, Nakatsu CH, Jones-Hall Y, Kozik A and Jiang Q: Vitamin E alpha- and gamma-tocopherol mitigate colitis, protect intestinal barrier function and modulate the gut microbiota in mice. Free Radic Biol Med 163: 180-189, 2021.
90. Wang Y, Shen W, Shi X, Fu F, Fan Y, Shen W, Cao Y, Zhang Q and Qi R: Alpha-tocopheryl succinate-conjugated G5 PAMAM dendrimer enables effective inhibition of ulcerative colitis. Adv Healthc Mater 6, 2017.
91. Zou Y, Li H, Graham ET, Deik AA, Eaton JK, Wang W, Sandoval-Gomez G, Clish CB, Doench JG and Schreiber SL: Cytochrome P450 oxidoreductase contributes to phospholipid peroxidation in ferroptosis. Nat Chem Biol 16: 302-309, 2020.
92. Yao X, Zhang Y, Hao J, Duan HQ, Zhao CX, Sun C, Li B, Fan BY, Wang X, Li WX, *et al*: Deferoxamine promotes recovery of traumatic spinal cord injury by inhibiting ferroptosis. Neural Regen Res 14: 532-541, 2019.
93. Guo Z, Lin J, Sun K, Guo J, Yao X, Wang G, Hou L, Xu J, Guo J and Guo F: Deferoxamine alleviates osteoarthritis by inhibiting chondrocyte ferroptosis and activating the Nrf2 pathway. Front Pharmacol 13: 791376, 2022.
94. Ma H, Wang X, Zhang W, Li H, Zhao W, Sun J and Yang M: Melatonin suppresses ferroptosis induced by high glucose via activation of the Nrf2/HO-1 signaling pathway in type 2 diabetic osteoporosis. Oxid Med Cell Longev 2020: 9067610, 2020.
95. Bai T, Li M, Liu Y, Qiao Z and Wang Z: Inhibition of ferroptosis alleviates atherosclerosis through attenuating lipid peroxidation and endothelial dysfunction in mouse aortic endothelial cell. Free Radic Biol Med 160: 92-102, 2020.
96. Rayatpour A, Foolad F, Heibatollahi M, Khajeh K and Javan M: Ferroptosis inhibition by deferiprone, attenuates myelin damage and promotes neuroprotection in demyelinated optic nerve. Sci Rep 12: 19630, 2022.
97. Liu X, Zhang J and Xie W: The role of ferroptosis in acute lung injury. Mol Cell Biochem 477: 1453-1461, 2022.
98. Millar AD, Rampton DS and Blake DR: Effects of iron and iron chelation in vitro on mucosal oxidant activity in ulcerative colitis. Aliment Pharmacol Ther 14: 1163-1168, 2000.
99. Minaiyan M, Mostaghel E and Mahzouni P: Preventive therapy of experimental colitis with selected iron chelators and anti-oxidants. Int J Prev Med 3 (Suppl 1): S162-S169, 2012.
100. Wu Y, Ran L, Yang Y, Gao X, Peng M, Liu S, Sun L, Wan J, Wang Y, Yang K, *et al*: Deferasirox alleviates DSS-induced ulcerative colitis in mice by inhibiting ferroptosis and improving intestinal microbiota. Life Sci 314: 121312, 2023.
101. Ghaith MM, El-Boshy M, Almasmoum H, Abdelghany AH, Azzeh FS, Almaimani RA, Idris S, Ahmad J, Mahbub AA, BaSalamah MA, *et al*: Deferasirox and vitamin D_3 co-therapy mitigates iron-induced renal injury by enhanced modulation of cellular anti-inflammatory, anti-oxidative stress, and iron regulatory pathways in rat. J Trace Elem Med Biol 74: 127085, 2022.
102. Chen X, Comish PB, Tang D and Kang R: Characteristics and biomarkers of ferroptosis. Front Cell Dev Biol 9: 637162, 2021.
103. Li YY, Wang XJ, Su YL, Wang Q, Huang SW, Pan ZF, Chen YP, Liang JJ, Zhang ML, Xie XQ, *et al*: Baicalein ameliorates ulcerative colitis by improving intestinal epithelial barrier via AhR/IL-22 pathway in ILC3s. Acta Pharmacol Sin 43: 1495-1507, 2022.
104. Thermozier S, Hou W, Zhang X, Shields D, Fisher R, Bayir H, Kagan V, Yu J, Liu B, Bahar I, *et al*: Anti-ferroptosis drug enhances total-body irradiation mitigation by drugs that block apoptosis and necroptosis. Radiat Res 193: 435-450, 2020.
105. Gounaris E, Heiferman MJ, Heiferman JR, Shrivastav M, Vitello D, Blatner NR, Knab LM, Phillips JD, Cheon EC, Grippo PJ, *et al*: Zileuton, 5-lipoxygenase inhibitor, acts as a chemopreventive agent in intestinal polyposis, by modulating polyp and systemic inflammation. PLoS One 10: e0121402, 2015.
106. Dächert J, Ehrenfeld V, Habermann K, Dolgikh N and Fulda S: Targeting ferroptosis in rhabdomyosarcoma cells. Int J Cancer 146: 510-520, 2020.
107. Shimada K, Skouta R, Kaplan A, Yang WS, Hayano M, Dixon SJ, Brown LM, Valenzuela CA, Wolpaw AJ and Stockwell BR: Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. Nat Chem Biol 12: 497-503, 2016.
108. Wang D, Peng Y, Xie Y, Zhou B, Sun X, Kang R and Tang D: Antiferroptotic activity of non-oxidative dopamine. Biochem Biophys Res Commun 480: 602-607, 2016.

109. Liu L, Wu Y, Wang B, Jiang Y, Lin L, Li X and Yang S: DA-DRD5 signaling controls colitis by regulating colonic M1/M2 macrophage polarization. Cell Death Dis 12: 500, 2021.
110. Kawano M, Saika K, Takagi R, Matsui M and Matsushita S: Tannic acid acts as an agonist of the dopamine D2L receptor, regulates immune responses, and ameliorates experimentally induced colitis in mice. Brain Behav Immun Health 5: 100071, 2020.
111. Wu Z, Geng Y, Lu X, Shi Y, Wu G, Zhang M, Shan B, Pan H and Yuan J: Chaperone-mediated autophagy is involved in the execution of ferroptosis. Proc Natl Acad Sci USA 116: 2996-3005, 2019.
112. Panés J, García-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, Dignass A, Nachury M, Ferrante M, Kazemi-Shirazi L, *et al*: Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: A phase 3 randomised, double-blind controlled trial. Lancet 388: 1281-1290, 2016.
113. Ocansey DKW, Pei B, Yan Y, Qian H, Zhang X, Xu W and Mao F: Improved therapeutics of modified mesenchymal stem cells: An update. J Transl Med 18: 42, 2020.
114. Ocansey DKW, Qiu W, Wang J, Yan Y, Qian H, Zhang X, Xu W and Mao F: The achievements and challenges of mesenchymal stem cell-based therapy in inflammatory bowel disease and its associated colorectal cancer. Stem Cells Int 2020: 7819824, 2020.
115. Ocansey DKW, Zhang Z, Xu X, Liu L, Amoah S, Chen X, Wang B, Zhang X and Mao F: Mesenchymal stem cell-derived exosome mitigates colitis via the modulation of the gut metagenomics-metabolomics-farnesoid X receptor axis. Biomater Sci 10: 4822-4836, 2022.
116. Wang G, Joel MDM, Yuan J, Wang J, Cai X, Ocansey DKW, Yan Y, Qian H, Zhang X, Xu W and Mao F: Human umbilical cord mesenchymal stem cells alleviate inflammatory bowel disease by inhibiting ERK phosphorylation in neutrophils. Inflammopharmacology 28: 603-616, 2020.
117. Ocansey DKW, Zhang L, Wang Y, Yan Y, Qian H, Zhang X, Xu W and Mao F: Exosome-mediated effects and applications in inflammatory bowel disease. Biol Rev Camb Philos Soc 95: 1287-1307, 2020.
118. Zhu Y, Qin H, Sun C, Shao B, Li G, Qin Y, Kong D, Ren S, Wang H, Wang Z, *et al*: Endometrial regenerative cell-derived exosomes attenuate experimental colitis through downregulation of intestine ferroptosis. Stem Cells Int 2022: 3014123, 2022.
119. Lin F, Chen W, Zhou J, Zhu J, Yao Q, Feng B, Feng X, Shi X, Pan Q, Yu J, *et al*: Mesenchymal stem cells protect against ferroptosis via exosome-mediated stabilization of SLC7A11 in acute liver injury. Cell Death Dis 13: 271, 2022.
120. Liu T, Jiang L, Tavana O and Gu W: The deubiquitylase OTUB1 mediates ferroptosis via stabilization of SLC7A11. Cancer Res 79: 1913-1924, 2019.
121. Shao C, Chen Y, Yang T, Zhao H and Li D: Mesenchymal stem cell derived exosomes suppress neuronal cell ferroptosis via lncGm36569/miR-5627-5p/FSP1 axis in acute spinal cord injury. Stem Cell Rev Rep 18: 1127-1142, 2022.
122. Sun Z, Wu J, Bi Q and Wang W: Exosomal lncRNA TUG1 derived from human urine-derived stem cells attenuates renal ischemia/reperfusion injury by interacting with SRSF1 to regulate ASCL4-mediated ferroptosis. Stem Cell Res Ther 13: 297, 2022.
123. Song Y, Wang B, Zhu X, Hu J, Sun J, Xuan J and Ge Z: Human umbilical cord blood-derived MSCs exosome attenuate myocardial injury by inhibiting ferroptosis in acute myocardial infarction mice. Cell Biol Toxicol 37: 51-64, 2021.
124. Tan Y, Huang Y, Mei R, Mao F, Yang D, Liu J, Xu W, Qian H and Yan Y: HucMSC-derived exosomes delivered BECN1 induces ferroptosis of hepatic stellate cells via regulating the xCT/GPX4 axis. Cell Death Dis 13: 319, 2022.
125. Atkuri KR, Mantovani JJ, Herzenberg LA and Herzenberg LA: N-acetylcysteine-a safe antidote for cysteine/glutathione deficiency. Curr Opin Pharmacol 7: 355-359, 2007.
126. Schmitt B, Vicenzi M, Garrel C and Denis FM: Effects of N-acetylcysteine, oral glutathione (GSH) and a novel sublingual form of GSH on oxidative stress markers: A comparative crossover study. Redox Biol 6: 198-205, 2015.
127. Ardite E, Sans M, Panés J, Romero FJ, Piqué JM and Fernández-Checa JC: Replenishment of glutathione levels improves mucosal function in experimental acute colitis. Lab Invest 80: 735-744, 2000.
128. Dong S, Lu Y, Peng G, Li J, Li W, Li M, Wang H, Liu L and Zhao Q: Furin inhibits epithelial cell injury and alleviates experimental colitis by activating the Nrf2-Gpx4 signaling pathway. Dig Liver Dis 53: 1276-1285, 2021.
129. Zhu D, Wu H, Jiang K, Xu Y, Miao Z, Wang H and Ma Y: Zero-valence selenium-enriched prussian blue nanozymes reconstruct intestinal barrier against inflammatory bowel disease via inhibiting ferroptosis and T cells differentiation. Adv Healthc Mater 12: e2203160, 2023.
130. Perry C, Kapur N and Barrett TA: DPP-4 as a novel biomarker for inflammatory bowel disease: Is it ready for clinical use? Inflamm Bowel Dis 26: 1720-1721, 2020.
131. Xie Y, Zhu S, Song X, Sun X, Fan Y, Liu J, Zhong M, Yuan H, Zhang L, Billiar TR, *et al*: The tumor suppressor p53 limits ferroptosis by blocking DPP4 activity. Cell Rep 20: 1692-1704, 2017.
132. Arab HH, Eid AH, Mahmoud AM and Senousy MA: Linagliptin mitigates experimental inflammatory bowel disease in rats by targeting inflammatory and redox signaling. Life Sci 273: 119295, 2021.
133. El-Ghannam MS, Saad MA, Nassar NN, El-Yamany MF and El-Bahy AAZ: Linagliptin ameliorates acetic acid-induced colitis via modulating AMPK/SIRT1/PGC-1α and JAK2/STAT3 signaling pathway in rats. Toxicol Appl Pharmacol 438: 115906, 2022.
134. Hu Y, Mao Z, Xu L, Yin L, Tao X, Tang Z, Qi Y, Sun P and Peng J: Protective effect of dioscin against intestinal ischemia/reperfusion injury via adjusting miR-351-5p-mediated oxidative stress. Pharmacol Res 137: 56-63, 2018.
135. Ozkan OV, Yuzbasioglu MF, Ciralik H, Kurutas EB, Yonden Z, Aydin M, Bulbuloglu E, Semerci E, Goksu M, Atli Y, *et al*: Resveratrol, a natural antioxidant, attenuates intestinal ischemia/reperfusion injury in rats. Tohoku J Exp Med 218: 251-258, 2009.
136. Stefanutti G, Pierro A, Parkinson EJ, Smith VV and Eaton S: Moderate hypothermia as a rescue therapy against intestinal ischemia and reperfusion injury in the rat. Crit Care Med 36: 1564-1572, 2008.
137. Meister AL, Doheny KK and Travagli RA: Necrotizing enterocolitis: It's not all in the gut. Exp Biol Med (Maywood) 245: 85-95, 2020.
138. Yin Y, Wu X, Peng B, Zou H, Li S, Wang J and Cao J: Curcumin improves necrotising microscopic colitis and cell pyroptosis by activating SIRT1/NRF2 and inhibiting the TLR4 signalling pathway in newborn rats. Innate Immun 26: 609-617, 2020.
139. Xu Y, Li X, Cheng Y, Yang M and Wang R: Inhibition of ACSL4 attenuates ferroptotic damage after pulmonary ischemia-reperfusion. FASEB J 34: 16262-16275, 2020.
140. Cui Y, Zhang Y, Zhao X, Shao L, Liu G, Sun C, Xu R and Zhang Z: ACSL4 exacerbates ischemic stroke by promoting ferroptosis-induced brain injury and neuroinflammation. Brain Behav Immun 93: 312-321, 2021.
141. Zhang L, Fan J, He J, Chen W, Jin W, Zhu Y, Sun H, Li Y, Shi Y, Jing Y, *et al*: Regulation of ROS-NF-κB axis by tuna backbone derived peptide ameliorates inflammation in necrotizing enterocolitis. J Cell Physiol 234: 14330-14338, 2019.
142. Tang B, Zhu J, Fang S, Wang Y, Vinothkumar R, Li M, Weng Q, Zheng L, Yang Y, Qiu R, *et al*: Pharmacological inhibition of MELK restricts ferroptosis and the inflammatory response in colitis and colitis-propelled carcinogenesis. Free Radic Biol Med 172: 312-329, 2021.