The Role of Ferroptosis in Inflammatory Bowel Disease: Mechanisms and Therapeutic Implications

Qiu Yan[1]

1 Hehai University

Funding: No specific funding was received for this work.

Potential competing interests: No potential competing interests to declare.

Abstract

Inflammatory Bowel Disease (IBD), encompassing Crohn's disease and ulcerative colitis, is characterized by chronic inflammation of the gastrointestinal tract, with an increasing incidence worldwide. Recent advancements in cellular biology have identified ferroptosis, a form of programmed cell death driven by iron-dependent lipid peroxidation, as a critical player in the pathology of IBD. This article reviews the current understanding of ferroptosis and its distinctive mechanisms, including the role of GPx4, Nrf2-HO-1 pathways, and iron metabolism in the context of IBD. It also examines the dual nature of iron in intestinal health and disease, contributing to both physiological functions and pathological processes through oxidative stress and inflammation. The implications of ferroptosis in the intestinal epithelial cell death, barrier function, and immune response are discussed, highlighting its potential as a novel therapeutic target. Despite the promising insights, the article underscores the need for further research to elucidate the complex mechanisms of ferroptosis in IBD and to translate these findings into effective therapeutic strategies. The emerging evidence positions ferroptosis at the crossroads of metabolic, inflammatory, and cell death pathways, offering a unique perspective on the interplay between nutrition, genetics, and immunity in intestinal health and disease.

Qiu Yan*

Faculty of Public Administration, Houhai University, 210098, Nanjing, China

***Corresponding Author:** Qiu Yan, Faculty of Public Administration, Houhai University, 210098, Nanjing, China; Email: qiuyan2003716@hhu.edu.cn

Keywords: Ferroptosis, Inflammatory Bowel Disease, Intestinal Epithelial Cells, Iron Metabolism, Oxidative Stress, GPX4, Nrf2-HO-1 Pathway, Therapeutic Targets.

1. Introduction

Ferroptosis, a novel manifestation of iron-dependent, lipid peroxidation-driven programmed cellular demise introduced in 2012, manifests hallmark traits inclusive of lipid peroxidation byproduct accrual and polyunsaturated fatty acid (PUFA) membrane depletion. Inflammatory bowel disease (IBD) delineates a constellation of obscure, nonspecific [1][2][3], chronic intestinal inflammatory ailments of idiopathic origin, encompassing ulcerative colitis (UC), Crohn's disease (CD), and indeterminate colitis. UC manifests as incessant colonic mucosal and submucosal inflammation, while CD exhibits sporadic involvement of the entire gastrointestinal tract, typically afflicting the terminal ileum, colon, and perianal regions [4][5][6][7][8]. Current conceptualizations attribute IBD pathogenesis to human environmental triggers, primarily entailing dysbiotic microbial flora precipitating unbridled activation of mucosal immunity in genetically predisposed hosts, thereby instigating persistent intestinal inflammation. Clinical manifestations primarily comprise diarrhea, abdominal discomfort, and, occasionally, hematochezia and mucopurulent stools.

Empirical investigations have delineated a correlative link between heightened iron consumption and augmented UC susceptibility and severity. Moreover, the degree of UC mucosal oxidative stress correlates directly with disease activity. Iron-chelating agents, serving as ferroptosis inhibitors, exhibit potential in ameliorating oxidative stress-induced reactive oxygen species (ROS) production and mitigating intestinal inflammation. This posits a plausible nexus between IBD and ferroptosis: excessive luminal iron deposition instigates ROS generation via the Fenton reaction, culminating in oxidative stress-induced lipid peroxidation and subsequent ferroptotic demise of intestinal epithelial cells (IECs), thereby undermining intestinal mucosal structural integrity and predisposing to IBD onset. Notably, elevated ferritin heavy chain (FTH) expression predominantly localizes within IECs in UC, suggesting preferential ferroptotic susceptibility in these cells. Additionally, transmission electron microscopy unveils mitochondrial atrophy within IECs, consonant with ferroptosis's morphological hallmarks [9][10][11].

IEC demise precipitates intestinal integrity disruption, impairing the physical mucosal barricade and compromising intestinal chemical, immunological, and biological defenses, thereby inciting a gamut of intestinal dysfunctions. Thus, a comprehensive elucidation of ferroptosis mechanics and its impact on IECs assumes paramount significance in IBD pathogenesis elucidation.

2. Ferroptosis and Its Mechanism

2.1. Overview of Ferroptosis

Ferroptosis stands as a distinct mode of cellular demise, demarcating itself from classical apoptotic and necrotic pathways. Its morphological hallmarks encompass mitochondrial condensation, augmented mitochondrial membrane density, and diminished or absent mitochondrial cristae. Biochemically, ferroptosis is characterized by iron accumulation, reactive oxygen species (ROS) generation, and excessive lipid peroxidation. Noteworthy involvement of specific genetic elements, including prostaglandin-endoperoxide synthase 2 (PTGS2), and acyl-CoA synthetase long-chain family member 4 (ACSL4), underscores the intricate orchestration of this phenomenon.

Ferroptosis inducers epitomize diverse categories: Firstly, glutathione (GSH) scavengers such as Erastin impede cystine/glutamate antiporter (system Xc-) activity, thereby depleting intracellular GSH reserves. Secondly, inhibitors of glutathione peroxidase 4 (GPX4), including RSL3, Altretamine, and DPI17, directly inhibit GPX4 function. Thirdly, FIN56 orchestrates GPX4 degradation whilst activating squalene synthase, thus depleting coenzyme Q10 (CoQ10) in the mevalonate pathway, compromising cellular antioxidant defenses and precipitating ferroptosis. Fourthly, FINO2, a lipophilic peroxide, selectively oxidizes labile intracellular iron, engendering extensive PUFA oxidation and concomitant GPX4 inhibition [12][13][14][15].

Ferroptosis inhibitors encompass diverse modalities: Iron chelators form complexes with iron ions, curtailing free intracellular iron pools and attenuating lipid peroxidation. β-mercaptoethanol counters Erastin-induced ferroptosis by fostering system Xc- activation through cysteine disulfide formation. Free radical scavenging antioxidants, exemplified by vitamin E and aromatic amine-based ferrostatin-1 (Fer-1) and liproxstatin-1 (Lip-1), intercept free radical propagation cascades, shielding lipids from auto-oxidation. Finally, lipoxygenase (LOX) inhibitors antagonize LOX-catalyzed lipid peroxidation, thereby mitigating ferroptotic progression.

2.2. Mechanism of Ferroptosis (Fig 1)

2.2.1. Pro-ferroptosis Mechanisms

Ferritin and Ferroptosis

Iron-dependent lipid peroxidation is one of the hallmark signals of ferroptosis. Fe^{3+} enters cells through the transferrin receptor (TFRC) and endocytosis, and after being reduced to Fe^{2+}, is assisted into the cytosolic labile iron pool (LIP) by divalent metal transporter 1 (DMT1). Ferritin serves as an inert storage form of iron and generally does not promote lipid peroxidation; however, when iron overload is severe, ferritin is hydrolyzed to Fe2+, enhancing the Fenton reaction and ROS production [16][17][18][19]. The intracellular Fe^{2+} is then stored in the LIP, increasing sensitivity to ferroptosis. Additionally, ferritin can increase sensitivity to ferroptosis by targeting autophagy, leading to the degradation of ferritin lysosomes and releasing iron into the LIP.

ACSL4 and Ferroptosis

ACSL4, a constituent of the ACSL lineage, orchestrates the enzymatic orchestration of acyl-CoA production in vivo, thus initiating the primary stage of lipid metabolism. Polyunsaturated fatty acid phospholipids (PUFA-PLs) undergo oxidation via both enzymatic and non-enzymatic routes. The enzymatic route, reliant upon lipoxygenase activity, entails the activation of ACSL4 and lysophosphatidylcholinyltransferase 3 (LPCAT3), pivotal in engendering lipid peroxides from PUFA-PLs during the process of ferroptosis [20][21][22][23]. Doll et al. elucidated ACSL4's role in facilitating the incorporation of long-chain PUFAs into membrane phospholipids, thereby rendering these membranous long-chain PUFAs predisposed to oxidation. Consequently, ACSL4 emerges as a critical agent in catalyzing ferroptosis by engaging in the biosynthesis of these readily oxidizable membrane phospholipids, rendering cells susceptible to ferroptosis inducers

such as RSL3. In neoplastic cells, radiotherapy induces heightened levels of reactive oxygen species (ROS) and augments ACSL4 expression, collectively fostering lipid peroxidation and culminating in ferroptotic demise of tumor cells, whereas ablation of the ACSL4 gene can confer substantial resistance to radiotherapy.

Endoplasmic Reticulum Stress and Ferroptosis

Protein kinase-like endoplasmic reticulum kinase (PERK) is a major sensor of endoplasmic reticulum stress. During the endoplasmic reticulum stress response, PERK is activated through phosphorylation, which in turn activates and regulates the eukaryotic initiation factor 2α subunit (eIF2α) and promotes the production of activating transcription factor 4 (ATF4) and C/EBP-homologous protein (CHOP) [24][25][26][27][28][29]. The PERK-eIF2α signaling pathway plays a role in cell ferroptosis by regulating the production of ROS. Preliminary studies with cells treated with RSL3 observed the occurrence of ferroptosis alongside the phosphorylation of eIF2α and upregulation of ATF4 and CHOP. Following this, the addition of the selective PERK inhibitor GSK 414 to the cells not only suppressed the expression of peIF2α, ATF4, and CHOP but also reduced the ferroptosis of cells stimulated by RSL3. These studies indicate the promoting role of endoplasmic reticulum stress in cell ferroptosis. Additionally, there is increasing evidence that the nuclear factor kappa-B (NF-κB) pathway may be involved in the regulation of endoplasmic reticulum stress signaling and cell ferroptosis.

Autophagy and Ferroptosis

The academic community currently defines ferroptosis as a type of autophagy-dependent cell death. Preliminary research has proven that autophagy promotes ferroptosis by degrading ferritin in fibroblasts and cancer cells. Knocking out autophagy-related genes Atg5 and Atg7 reduced lipid peroxidation and intracellular Fe2+ levels, limiting Erastin-induced cell ferroptosis. Additionally, Atg5-mediated autophagy is essential for ferritin degradation, and the nuclear receptor coactivator 4 (NCOA4) acts as a selective transport receptor for ferritin autophagy conversion. The genetic inhibition of NCOA4 weakens the degradation of ferritin and subsequent ferroptosis. Lipophagy, a selective form of autophagy, promotes lipid peroxidation by mediating the autophagic degradation of intracellular lipid droplets, inducing cell ferroptosis, providing new evidence for the link between autophagy and ferroptosis.

Immune Cells and Ferroptosis

In recent years, cancer research has discovered that CD8+ T lymphocytes can suppress tumors by inducing ferroptosis and necroptosis. Specifically, immunotherapy activates CD8+ T lymphocytes to release interferon-γ (IFNγ), which downregulates the expression of solute carrier family 3 member 2 (SLC3A2) and solute carrier family 7 member 11 (SLC7A11), restricting the uptake of cystine by tumor cells, thereby promoting lipid peroxidation and subsequent ferroptosis in tumor cells, aiding in anti-tumor effects.

2.2.2. Mechanisms Inhibiting Ferroptosis

The GPX4-GSH Pathway and Ferroptosis

Ferroptosis is a form of cell death caused by lipid peroxidation. The selenium-containing enzyme Glutathione Peroxidase 4 (GPX4) was the first discovered central inhibitor in ferroptosis, uniquely capable of catalyzing the reduction of oxidized biomolecules using glutathione (GSH), a tripeptide containing a thiol group and an indispensable cofactor for GPX4. GPX4 uses GSH to eliminate lipid peroxides, protecting cells from ferroptosis. Being a selenoprotein, the delivery of selenium to animals or cells can inhibit ferroptosis, indicating that selenium can affect the sensitivity to ferroptosis.

The FSP1-CoQ10 Pathway and Ferroptosis

The mevalonate pathway-derived coenzyme Q10 (CoQ10) is not only a key component of the mitochondrial electron transport chain but also inhibits lipid peroxidation by suppressing free radical intermediates outside the mitochondria, hence the activation of CoQ10 can suppress ferroptosis. It has been reported that in the absence of GPX4, ferroptosis suppressor protein 1 (FSP1) can also block lipid peroxidation and inhibit ferroptosis by regenerating reduced CoQ10, providing a new independent FSP1-CoQ10 pathway against ferroptosis.

GCH1-BH4-Phospholipid Pathway and Ferroptosis

This pathway consists of guanosine triphosphate cyclohydrolase 1 (GCH1) and its metabolic derivative tetrahydrobiopterin (BH4). Recent literature reports that cells expressing GCH1 inhibit ferroptosis by synthesizing BH4, which causes lipid remodeling and selectively prevents the consumption of two PUFAs tails in phospholipids. Lipidomics analysis found that cells expressing GCH1 can also regenerate reduced CoQ10 after induction; similarly, in their experiments, GCH1 was overexpressed in GPX4-knockout cells and primarily protected cells from ferroptosis through the antioxidative actions of BH4, proving that this is a pathway completely independent of GPX4-mediated ferroptosis inhibition.

Keap1-Nrf2 Pathway and Ferroptosis

Nuclear transcription factor E2-related factor 2 (Nrf2) is a key transcription factor in cellular antioxidant response, with its targets playing significant roles in iron metabolism and lipid metabolism. Kelch-like ECH-associated protein 1 (Keap1) is the specific receptor for Nrf2, which, upon oxidative stress, changes conformation, does not interact with Nrf2, thus preventing its degradation [30][31][32][33]. The accumulated Nrf2 then moves to the nucleus and promotes transcription of related genes, such as glutamate-cysteine ligase modified subunit (GCLM) and GPXs, thereby enhancing system Xc-. Nrf2 upregulates ferritin transcription genes, increases iron storage, and reduces labile iron to regulate iron homeostasis, and also alters the expression of ferroportin-1 (FPN1) to change labile iron entry and exit from cells. Therefore, Nrf2 activation can balance cellular iron overload situations and prevent oxidative stress. Activated Nrf2 can also upregulate GPXs to improve cellular lipid peroxidation; baicalein has been shown to alleviate ferroptosis by preventing Nrf2 degradation and raising GPX4 levels. Furthermore, Nrf2 exerts protective effects against ferroptosis through the induced expression of iron-related target genes such as heme oxygenase-1 (HO-1), metallothionein (MT-1G), and FTH1. However, contradictorily, studies have shown that overactivation of the NRF2-HO-1 pathway can interfere with iron ion metabolism leading to ferroptosis.

NF-κB Pathway and Ferroptosis

The NF-κB pathway plays a key role in regulating cell survival and proliferation. In human breast cancer cells, the phosphorylation of the nuclear factor kappa B p65 subunit (NF-κB p65) inhibited PERK-mediated ferroptosis, while inhibition of the NF-κB signaling pathway led to cellular ferroptosis, as seen in IECs. In liver cancer cells, the activation of NF-κB led to the upregulation of the iron chelator LCN2, which by depleting iron, inhibited ferroptosis. Additionally, the ferroptosis inducer Erastin can control the development of sepsis by inhibiting the NF-κB pathway [34][35][36][37]. However, in glioblastoma cells, NF-κB activation promotes ferroptosis by downregulating ATF4 expression, which seems contradictory to previous studies, hence the specific mechanism of the NF-κB pathway needs further clarification.

System Xc- and Ferroptosis

System Xc- is composed of SLC7A11 and SLC3A2. The conversion of cystine (Cys-Cys) to cysteine (Cys) in cells is crucial for the synthesis of GSH, which reduces ROS and reactive nitrogen species to decrease cellular oxidative damage and avoid ferroptosis. However, high levels of glutamate can inhibit system Xc-, leading to insufficient Cys-Cys absorption, further causing a shortage of Cys in cells, depleting the antioxidative GSH, and leading to the inactivation of GSH-dependent peroxidase GPX4, resulting in the accumulation of lipid peroxides and inducing ferroptosis. Moreover, Erastin can promote ferroptosis by inhibiting the activity of system Xc- through binding with Solute carrier family 7 member 5 (SLC7A5).

3. The Mechanism of Ferroptosis in IBD on IECs

3.1. The Mechanism of GPx4-Regulated Ferroptosis in IECs in IBD

Previous studies have observed the suppression of the key antioxidative enzyme GPX4 in mRNA and protein levels when UC is induced in mice with DSS, while shenmaoside ameliorated this suppression and intestinal inflammation [38][39][40]. Furin, also known as a proprotein convertase, is reported in a high-quality experimental study that its loss in CD4+ T lymphocytes can cause severe spontaneous colitis, revealing that GPX4 protects against PUFA-stimulated lipid peroxidation in intestinal epithelial cells. However, the death of IECs is not a prerequisite for intestinal inflammation in mice lacking a Gpx4 allele; they believe that perhaps one Gpx4 allele is enough to prevent the ferroptosis of ICE but insufficient to prevent the production of inflammatory factors induced by PUFAs. Similarly, a significant decrease in Gpx4 levels was also observed in the colon tissues of CD patients.

3.2. The Mechanism of Nrf2-HO-1 Pathway Regulated Ferroptosis in IECs in IBD

As mentioned above, Nrf2 is a key factor in inhibiting ferroptosis, but its excessive activation may lead to ferroptosis. Previous studies have found that the expression of Nrf2 and HO-1 increases in UC mice induced by DSS while the ferroptosis inhibitor Fer-1 reverses the expression of Nrf2 and HO-1 and significantly upregulates the expression of GPX4

and FTH1 in colonic IECs, inhibiting the expression of cyclooxygenase-2 (COX-2) and ACSL4. This fully demonstrates that ferroptosis may act on UC epithelial cells through the Nrf2-HO-1 pathway. Astragalus polysaccharides have been proven to significantly improve UC, and similarly, they can reduce the expression of Nrf2 and HO-1 in the colonic tissues of mice after DSS stimulation, reduce the expression of ferroptosis-related factors PTGS2, FTH, and FTL, and restore the levels of ferroptosis markers MDA, GSH, and iron overload to steady state in a dose-dependent manner, and inhibit ferroptosis in human Caco-2 cells. Deferoxamine is a chelator that can be used to treat acute iron poisoning; previous studies have found that treating UC mice induced by DSS with deferoxamine not only regulates the release of inflammatory factors but also promotes the expression of the antioxidative proteins Keap-1 and HO-1 in the Nrf-2 pathway in colonic tissue to improve inflammation.

3.3. Endoplasmic Reticulum Stress Regulated Ferroptosis in IECs in IBD

Endoplasmic reticulum stress has been proven to exacerbate intestinal inflammation, and endoplasmic reticulum stress in either lymphocytes or IECs can cause intestinal dysfunction, leading to or exacerbating inflammatory diarrhea. Selective inhibition of the key stress sensor of endoplasmic reticulum stress signals—PERK, can significantly reduce IEC ferroptosis and improve experimental colitis, while the specific absence of NF-κB p65 in IECs exacerbates DSS-induced UC in mice through endoplasmic reticulum stress-mediated IEC ferroptosis.

4. The Role of Iron in IBD

Iron is typically absorbed in the small intestine through an active transport process. After food enters the intestine, the transferrin inside the intestinal mucosal cells binds with the iron in the food, and then it binds to the transferrin receptor (TFRC) on the microvilli of the intestinal mucosa to enter the intestinal epithelial cells. Excessive oral iron intake in the intestines can produce reactive oxygen species (ROS) through the Fenton reaction and Haber-Weiss reaction, thereby triggering oxidative stress and leading to a series of intestinal diseases. Firstly, the excessive iron participates in the Fenton reaction causing lipid peroxidation of intestinal cells; secondly, it can cause mitochondrial damage within cells; thirdly, excess iron can induce endoplasmic reticulum stress and exacerbate intestinal inflammation; finally, it can also disrupt the balance of intestinal flora by reducing the growth of probiotics through the decrease of short-chain fatty acids.

Early studies believed that IBD could cause iron deficiency anemia due to malabsorption, and clinically, oral iron has been used to treat iron deficiency anemia in IBD. However, excessive iron intake can lead to intestinal iron overload, causing ROS imbalance and disturbing the intestinal flora, thereby worsening IBD. Hfe gene latent mutations lead to increased proximal intestinal iron absorption; colonic malondialdehyde (MDA) levels rise in Hfe knockout mice, making them more susceptible to experimental colitis. In addition, increased expression of ferritin heavy chain (FTH) and ferritin light chain (FTL) has also been observed in the colonic tissues of mice with dextran sulfate sodium (DSS)-induced experimental colitis. These emphasize the pathological role of iron overload in colitis. However, there is a contradiction, as previous studies have reported that oral iron chelators can improve experimental colitis in rats, but oral iron given to young mice prevented the dysbiosis of the intestinal flora and the occurrence of colitis.

5. Discussion

The incidence of IBD is increasing worldwide, and although its exact etiology remains unclear, widespread and uncontrolled death of intestinal epithelial cells (IECs) can be observed in both human patients and animal models of the intestinal mucosa. This type of cell death can exacerbate inflammation by disrupting the tight junctions of the intestinal barrier, leading to overactivation of the intestinal immune system, excessive secretion of pro-inflammatory cytokines and chemokines, thereby causing secondary damage to the intestinal mucosa. This creates a vicious cycle.

Ferroptosis is a newly discovered form of programmed cell death that has been extensively studied in the context of cancer and neurological diseases, as well as ischemia/reperfusion, but not much in intestinal research. For example, in colorectal cancer, Erastin can induce apoptosis in rectal cancer cells, but its specific mechanism is unclear. In intestinal inflammation, the expression of ACSL4 and PTGS2 is upregulated in the colonic tissues of colitis mice; P53 has a higher mutation rate in patients with chronic UC, and mice with P53 gene knockout exhibit histopathological changes indicative of inflammation in the intestine; UC patients often lack folate due to absorption issues, and taking folate or its metabolic precursors can alleviate colitis-related tissue damage. However, whether these effects improve or exacerbate intestinal inflammation through the regulation of ferroptosis and the specific mechanisms involved require further research. More directly, certain potent ferroptosis inhibitors such as iron chelators, Fer-1, and Lip-1 can alleviate colitis-related intestinal damage, whereas pro-ferroptosis factors like iron and dietary PUFAs can exacerbate intestinal inflammation.

Importantly, the use of adalimumab and infliximab, which are widely used to treat moderate to severe IBD, does not improve the inflammation in colitis mice worsened by oral iron; the partial effect of 5-aminosalicylic acid treatment for IBD is exerted through upregulating HO-1; furthermore, anti-inflammatory drugs used for treating UC, such as sulfasalazine, may induce ferroptosis by inhibiting the function of system Xc-. These suggest the irreplaceable value of regulating ferroptosis-related pathways in treating IBD. This underlines the crucial role of ferroptosis in IBD to some extent, but current research is far from elucidating its specific mechanisms. In addition, studies on ferroptosis in intestinal diseases have focused on IECs, but the impact of the same factors on ferroptosis and their mechanisms may differ in different environments and different cells. Whether immune cells, key to the development and progression of IBD, undergo ferroptosis in intestinal injury remains unclear. Therefore, more extensive and profound research is needed to further elucidate the significance of ferroptosis in the pathogenesis of IBD and other intestinal diseases.

6. Conclusion

The exploration of ferroptosis in the context of IBD presents a novel and significant avenue for understanding and treating this complex condition. The intricate interplay between iron metabolism, intestinal epithelial cell integrity, and immune responses highlights ferroptosis not just as a cellular event but as a potential therapeutic target. The dual role of iron as both a vital nutrient and a facilitator of oxidative stress underscores the delicate balance required in managing IBD. The emerging evidence suggests that modulating ferroptosis pathways could offer new strategies for mitigating intestinal inflammation and restoring mucosal health. However, the precise mechanisms by which ferroptosis influences IBD

pathology remain to be fully elucidated, demanding further investigation. As research progresses, it is imperative to develop a deeper understanding of the molecular underpinnings of ferroptosis in IBD, which will pave the way for innovative treatments that address the root causes of the disease rather than merely alleviating symptoms.

Statements and Declarations

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding

This study was not funded

Acknowledgements

We acknowledge the editors and reviewers for their helpful suggestions on this paper.

References

1. ^*Chen H, Wang W, Xu C, et al. A novel SOS1-ALK fusion variant in a patient with metastatic lung adenocarcinoma and a remarkable response to crizotinib[J]. Lung Cancer, 2020, 142: 59-62.*
2. ^*Zhou Y, Lizaso A, Mao X, et al. Novel AMBRA1-ALK fusion identified by next-generation sequencing in advanced gallbladder cancer responds to crizotinib: a case report[J]. Annals of Translational Medicine, 2020, 8(17).*
3. ^*Villa M, Sharma G G, Manfroni C, et al. New advances in liquid biopsy technologies for anaplastic lymphoma kinase (ALK)—Positive cancer[J]. Cancers, 2021, 13(20): 5149.*
4. ^*Ma Y S, Liu J B, Wu T M, et al. New therapeutic options for advanced hepatocellular carcinoma[J]. Cancer Control, 2020, 27(3): 1073274820945975.*
5. ^*Wu X, Li J, Gassa A, et al. Circulating tumor DNA as an emerging liquid biopsy biomarker for early diagnosis and therapeutic monitoring in hepatocellular carcinoma[J]. International journal of biological sciences, 2020, 16(9): 1551.*
6. ^*Valery M, Vasseur D, Fachinetti F, et al. Targetable Molecular Alterations in the Treatment of Biliary Tract Cancers: An Overview of the Available Treatments[J]. Cancers, 2023, 15(18): 4446.*
7. ^*Tümen D, Heumann P, Gülow K, et al. Pathogenesis and current treatment strategies of hepatocellular carcinoma[J]. Biomedicines, 2022, 10(12): 3202.*

8. ^Hewitt D B, Aziz H, Brown Z J, et al. Role of genetic testing in hepatic, pancreatic, and biliary cancers[J]. Surgical Oncology, 2022: 101844.
9. ^Bekaii-Saab T S, Bridgewater J, Normanno N. Practical considerations in screening for genetic alterations in cholangiocarcinoma[J]. Annals of Oncology, 2021, 32(9): 1111-1126.
10. ^Reig M, Forner A, Ávila M A, et al. Diagnosis and treatment of hepatocellular carcinoma. Update of the consensus document of the AEEH, AEC, SEOM, SERAM, SERVEI, and SETH[J]. Medicina Clínica (English Edition), 2021, 156(9): 463. e1-463. e30.
11. ^Thamlikitkul L, Parinyanitikul N, Sriuranpong V. Genomic medicine and cancer clinical trial in Thailand[J]. Cancer Biology & Medicine, 2024, 21(1): 10.
12. ^You Y, Chen Y, Zhang Y, Zhang Q, Yu Y, Cao Q. Mitigation role of physical exercise participation in the relationship between blood cadmium and sleep disturbance: a cross-sectional study. BMC Public Health. 2023;23(1):1465.
13. ^You Y, Chen Y, You Y, Q Zhang, Q Cao. Evolutionary Game Analysis of Artificial Intelligence Such as the Generative Pre-Trained Transformer in Future Education. Sustainability, 2023, 15(12): 9355.
14. ^You Y, Chen Y, Li J, Zhang Q, Zhang Y, Yang P, Cao Q. Physical activity mitigates the influence of blood cadmium on memory function: a cross-sectional analysis in US elderly population. Environmental Science and Pollution Research. 2023;30(26):68809-68820.
15. ^You Y, Chen Y, Chen X, Wei M, Yin J, Zhang Q, Cao Q. Threshold effects of the relationship between physical exercise and cognitive function in the short-sleep elder population. Frontiers in Aging Neuroscience. 2023;15:1214748.
16. ^Cao Q, Zhang Q, Chen YQ, Fan AD, Zhang XL. Risk factors for the development of hepatocellular carcinoma in Chengdu: a prospective cohort study. European Review for Medical and Pharmacological Sciences. 2022;26(24):9447-9456.
17. ^Cao Q, Zhang Q, Li XC, Ren CF, Qiang Y. Impact of sleep status on lung adenocarcinoma risk: a prospective cohort study. European Review for Medical and Pharmacological Sciences. 2022;26(20):7641-7648.
18. ^You Y, Chen Y, Zhang Q, Yan N, Ning Y, Cao Q. Muscle quality index is associated with trouble sleeping: a cross-sectional population based study. BMC Public Health. 2023;23(1):489.
19. ^Cao Q, Zhang Q, Zhou KX, Li YX, Yu Y, He ZX, Xiang ZB, Guan HR, Zhen JC, Lin RT, Liao YJ, Qiang Y, Li XC. Lung cancer screening study from a smoking population in Kunming. European Review for Medical and Pharmacological Sciences. 2022;26(19):7091-7098.
20. ^Qiang C, Qi Z, Yi Q. Mechanisms of p2x7 receptor involvement in pain regulation: a literature review. Acta Medica Mediterranea, 2022, 38(2): 1187-1194.
21. ^Cao Q, Zhang Q, Chen Y Q, Li J X, Yi Q. The Association Between Unhealthy Weight Loss Behaviours and Depressive Symptoms in Adolescents: a Cross-Sectional Study. Advances in Education, Humanities and Social Science Research, 2022, 1(3): 237-237.
22. ^Cao Q, Zhang Q, Chen Y, He Z, Xiang Z, Guan H, Yan N, Qiang Y, Li M. The relationship between non-suicidal self-injury and childhood abuse in transgender people: a cross-sectional cohort study. Frontiers in psychology, 2023, 14: 1062601.
23. ^You Y, Chen Y, Zhang Q, Hu X, Li X, Yang P, Zuo Q, Cao Q. Systematic and meta-based evaluation of the

relationship between the built environment and physical activity behaviors among older adults. PeerJ, 2023, 11: e16173.

24. ^*Cao Q, Ye X, Wu X, Zhang Q, Gong J, Chen Y, You Y, Shen J, Qiang Y, Cao G. Therapeutic efficacy of rare earth carbonate with chemoradiotherapy in late-stage non-small cell lung cancer: a cohort prospective study. Frontiers in Endocrinology, 2023, 14: 1301032.*
25. ^*Cao Q, Zhu J, Wu X, Li J, Chen Y, You Y, Li X, Huang X, Zhang Y, Li R, Han D. Efficacy and Safety Assessment of Intrathoracic Perfusion Chemotherapy Combined with immunological factor Interleukin-2 in the Treatment of Advanced Non-Small Cell Lung Cancer: A Retrospective Cohort Study. J Cancer 2024; 15(7):2024-2032.*
26. ^*Cao Q, Wu X, Zhang Q, Gong J, Chen Y, You Y, Shen J, Qiang Y, Cao G. Mechanisms of action of the BCL-2 inhibitor venetoclax in multiple myeloma: a literature review. Frontiers in Pharmacology, 2023, 14: 1291920.*
27. ^*Cao Q, Wu X, Chen Y, Wei Q, You Y, Qiang Y, Cao G. The impact of concurrent bacterial lung infection on immunotherapy in patients with non-small cell lung cancer: a retrospective cohort study. Frontiers in cellular and infection microbiology, 2023, 13: 1257638.*
28. ^*Wu X, Zhou Z, Cao Q, Chen Y, Gong J, Zhang Q, Qiang Y, Lu Y, Cao G. Reprogramming of Treg cells in the inflammatory microenvironment during immunotherapy: a literature review. Frontiers in immunology, 2023, 14: 1268188.*
29. ^*You Y, Chen Y, Wang X, Wei M, Zhang Q, Cao Q. Accelerometer-measured physical activity patterns are associated with phenotypic age: Isotemporal substitution effects. Heliyon, 2023, 9(9):e19158.*
30. ^*Cao Q, Wang Q, Wu X, Zhang Q, Huang J, Chen Y, You Y, Qiang Y, Huang X, Qin R, Cao G. A literature review: mechanisms of antitumor pharmacological action of leonurine alkaloid. Frontiers in pharmacology, 2023, 14: 1272546.*
31. ^*You Y, Wei M, Chen Y, Fu Y, Ablitip A, Liu J, Ma X. The association between recreational physical activity and depression in the short sleep population: a cross-sectional study. Frontiers in Neuroscience, 2023, 17: 1016619.*
32. ^*Chen YQ, Zhu XL, You YW, Zhang Q, Dai T. Evaluation of status quo and determinants of catastrophic health expenditure among empty-nest elderly in China: evidence from the China health and retirement longitudinal survey (CHARLS). European review for medical and pharmacological sciences, 2023, 27(4): 1398-1412.*
33. ^*Chen YQ, You YW, Zhang Q, Wang YD, Dai T. Systematic evaluation of influencing factors for Chinese rural doctors' job satisfaction and turnover intention: based on the two-factor theory. European review for medical and pharmacological sciences, 2022, 26(18): 6469-6486.*
34. ^*Chen Y, You Y, Wang Y, Wang Y, Dai T. Global insights into rural health workers' job satisfaction: a scientometric perspective. Frontiers in public health, 2022, 10: 895659.*
35. ^*Zheng HQ, Ma YC, Chen YQ, Xu YY, Pang YL, Liu L. Clinical analysis and risk factors of bronchiolitis obliterans after Mycoplasma Pneumoniae pneumonia. Infection and Drug Resistance, 2022: 4101-4108.*
36. ^*Xu J, Chen Y, Yue M, Yu J, Han F, Xu L, Shao Z. Prevalence of Neisseria meningitidis serogroups in invasive meningococcal disease in China, 2010-2020: a systematic review and meta-analysis. Human Vaccines & Immunotherapeutics, 2022, 18(5): 2071077.*
37. ^*Chen Y, You Y, Wang Y, Wang Y, Dai T. Systematic and meta-based evaluation on job satisfaction of village doctors: An urgent need for solution issue. Frontiers in Medicine, 2022, 9: 856379.*

38. ^*Zheng H, Yu X, Chen Y, Lin W, Liu L. Effects of Inhaled Corticosteroids on Lung Function in Children With Post-infectious Bronchiolitis Obliterans in Remission. Frontiers in Pediatrics, 2022, 10: 827508.*
39. ^*You Y, Chen Y, Fang W, Li X, Wang R, Liu J, Ma X. The association between sedentary behavior, exercise, and sleep disturbance: A mediation analysis of inflammatory biomarkers. Frontiers in Immunology, 2023, 13: 1080782.*
40. ^*Hu X, Chen Y, Shen Y, Tian R, Sheng Y, Que H. Global prevalence and epidemiological trends of Hashimoto's thyroiditis in adults: A systematic review and meta-analysis. Frontiers in Public Health, 2022, 10: 1020709.*