Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

Cuproptosis Cell Death Molecular Events and Pathways to Liver Disease

Yun Mao[1,*], Huilan Chen[1,*], Weihan Zhu[2], Shunlan Ni[3], Shengnan Luo[3], Shiyue Tang[3], Zhiyi Chen[4], Qin Wang[3], Jinxian Xu[3], Qi Tu[5], Haijun Chen[3,*], Lujian Zhu[3,*]

[1]Department of Gerontology, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, People's Republic of China; [2]The Second Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou, People's Republic of China; [3]Department of Infectious Disease, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, People's Republic of China; [4]Department of Gastroenterology, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, People's Republic of China; [5]Department of Neurosurgery, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, People's Republic of China

*These authors contributed equally to this work

Correspondence: Lujian Zhu; Haijun Chen, Department of Infectious Disease, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, People's Republic of China, Email zhulj_brave@163.com; 19564367335@163.com

Abstract: Chronic liver disease ranks as the 11th leading cause of death worldwide, while hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related mortality, representing a substantial risk to public health. Over the past few decades, the global landscape of chronic liver diseases, including hepatitis, metabolic dysfunction-associated steatotic liver disease (MASLD), liver fibrosis, and HCC, has undergone substantial changes. Copper, a vital trace element for human health, is predominantly regulated by the liver. Both copper deficiency and excess can lead to cellular damage and liver dysfunction. Copper deposition is a genetic process of copper-dependent cell death associated with mitochondrial respiration, which is associated with cardiovascular disease and IBD. However, the roles of copper overload and cuproptosis in liver disease remain largely underexplored. This article examines recent studies on copper metabolism and cuproptosis in chronic liver disease, investigating the potential of targeting copper ions as a therapeutic approach. The objective is to offer insights and guidance for future investigations in this developing field of study.

Keywords: copper metabolism, cuproptosis, mechanisms, liver diseases, treatment

Introduction

Liver diseases encompass pathological changes in the liver caused by a variety of internal and external factors, significantly impairing the organ's normal physiological functions. A wide range of factors can contribute to liver diseases, including drugs, chemical agents, viral infections, excessive alcohol consumption, malnutrition, and acid-base imbalances.[1,2] Based on their diverse etiologies and pathogenesis, liver diseases are categorized into acute liver injury (ALI), viral hepatitis, alcoholic liver disease (ALD), MASLD, liver fibrosis, cirrhosis, and HCC.[3] In recent years, changes in living environments and irregular lifestyles have led to a growing global prevalence of liver diseases, making them a significant public health challenge. Epidemiological data reveal that end-stage liver diseases, such as viral hepatitis, cirrhosis, and HCC, result in approximately 2 million deaths annually worldwide.[4] Therefore, understanding the pathogenesis of liver diseases and developing targeted therapeutic drugs are of critical importance for advancing clinical treatments and improving patient outcomes.

Programmed cell death (PCD) is essential for the growth, development, and survival of multicellular organisms, playing a critical role in maintaining dynamic homeostasis and overall organismal health.[5] The primary forms of programmed cell death include apoptosis, necroptosis, pyroptosis, ferroptosis, autophagy, and the recently identified cuproptosis.[6] The most researched of them has been apoptosis, while cuproptosis has not been thoroughly or precisely examined.[7] Cuproptosis is a newly recognized type of cell death that is characterized by its reliance on copper, the accumulation of lipid-acylated proteins, and the depletion of iron-sulfur (Fe-S) cluster proteins, distinguishing it from

Received: 2 October 2024
Accepted: 7 January 2025
Published: 20 January 2025

other regulated cell deaths (RCDs).[8] This type of cell death is triggered when excess copper causes the aberrant oligomerization of copper-dependent lipoylated proteins in the tricarboxylic acid (TCA) cycle, leading to a reduction in Fe-S cluster protein levels.[9,10]

Copper is an essential trace element with unique redox properties due to its ability to alternate between Cu^{1+} and Cu^{2+} oxidation states, which are crucial for various physiological processes.[11,12] Copper serves as a cofactor for several enzymes, including cytochrome c oxidase, which is crucial for cellular respiration and energy synthesis.[13] Beyond its role as an enzymatic cofactor, copper also regulates cell growth and function.[14] Insufficient copper intake can hinder growth and development; in contrast, excessive copper levels may lead to oxidative stress, cell death, and tissue damage.[11] The human body regulates copper levels through a dynamic equilibrium, and any disruption of this balance may lead to adverse health consequences.[15] Disorders of copper homeostasis, whether hereditary or acquired,[16] can contribute to diseases such as Menkes disease,[17] Wilson's disease (WD),[18] and cancer.[19] Hence, maintaining appropriate copper levels is crucial for human health.[20] Recent studies suggest that disturbances in copper homeostasis may result in cellular damage via several mechanisms, playing a role in the development of various liver diseases.[21] This review attempts to clarify the mechanisms behind cuproptosis and its role in chronic liver disorders, presenting new insights for the therapeutic management and therapy of these conditions.

Definition and Characteristics of Cuproptosis

In March 2022, Tsvetkov et al identified a novel form of PCD called "cuproptosis", characterized by the toxic accumulation of intracellular copper ions, resulting in metabolic dysfunction and subsequent cell death.[22] In contrast to conventional cell death mechanisms like apoptosis, necrosis, and autophagy, cuproptosis is distinctly characterized by its dependence on copper ions to interfere with cellular metabolic pathways.[23] This mechanism specifically entails the abnormal accumulation of copper ions within cells, predominantly affecting and binding to lipoylated proteins in the TCA cycle.[9] This interaction results in the aggregation of these proteins, impairing their function and causing a reduction in Fe-S cluster proteins, essential for cellular respiration and energy production. The resultant proteotoxic stress and metabolic dysregulation induce a unique type of cell death, differentiating cuproptosis from other well-defined mechanisms.[22]

Comparison of Cuproptosis with Other Modes of Cell Death

Cuproptosis is a unique mode of cell death that differs from other forms such as ferroptosis, apoptosis, autophagy, necrosis, and pyroptosis, both in its underlying mechanism and physiological implications. Ferroptosis is another recently identified type of cell death, similar to cuproptosis. However, cuproptosis is caused by the toxic accumulation of copper ions, whereas ferroptosis is propelled by iron-dependent lipid peroxidation of unsaturated fatty acids in the cell membrane, which is mediated by ferrous ions and ester oxygenase.[24] Cuproptosis is caused by copper ions that produce significant numbers of reactive oxygen species (ROS) through Fenton-like processes, in contrast to ferroptosis, which is mostly linked to iron. Oxidative damage, DNA degradation, lipid peroxidation, protease activity inhibition, and cellular growth suppression are all caused by this formation of ROS. Furthermore, copper ions attach to proteins that are esteroylated, which hinders mitochondrial metabolism and encourages cuproptosis.[25] In contrast, apoptosis, another form of PCD, is governed by a cascade of intracellular signaling pathways, such as the activation of caspases and the release of cytochrome c from mitochondria.[26] Apoptosis involves a systematic and regulated breakdown of cellular components, whereas cuproptosis arises from metabolic impairment caused by excessive accumulation of intracellular copper, resulting in a unique type of cellular dysfunction.[27] Autophagy is a survival mechanism activated during cellular stress, facilitating the degradation and recycling of intracellular components to uphold cellular homeostasis. In contrast to cuproptosis, which induces cell death by disrupting metabolic processes, autophagy generally functions as a protective mechanism that safeguards against cell death in unfavorable conditions. Under specific conditions, dysregulated autophagy may lead to cell death, differentiating it from the copper-induced metabolic failure observed in cuproptosis.[28] Necrosis is a form of cell death characterized by the unregulated breakdown of cellular components resulting from external injury, frequently eliciting a significant inflammatory response. In contrast, cuproptosis represents a regulated process that selectively impacts specific cell types, particularly those showing elevated mitochondrial activity,

such as tumor cells.[29] This selective nature of cuproptosis highlights its potential as a therapeutic target in cancer treatment. Pyroptosis, unlike cuproptosis, is a pro-inflammatory form of PCD mediated by the activation of Gasdermin family proteins, leading to pore formation in the plasma membrane and the release of pro-inflammatory cytokines like IL (interleukin) −1β and IL-18. Pyroptosis is crucial in the host's response to infections and relies on the activation of caspase-1. In contrast, cuproptosis is characterized by metabolic disturbances resulting from copper toxicity, rather than inflammatory cascades.[30] Cuproptosis, although identified recently, has attracted significant attention due to its unique mechanism and potential implications in cancer therapy, necessitating further investigation to clarify its role in disease pathogenesis and treatment. The cell death pathways are summarized in Table 1.

Molecular Mechanisms Associated with Cuproptosis

Oxidative Stress

Cells maintain a balance between oxidative processes and antioxidant defenses under normal physiological conditions. Disruption of this equilibrium induces oxidative stress, potentially resulting in cellular damage and contributing to the onset of various diseases.[31] The Fenton reaction, a metal-catalyzed process, is a significant contributor to oxidative stress due to its production of considerable amounts of ROS.[32] Copper ions play a significant role in this reaction by catalyzing the conversion of hydrogen peroxide (H_2O_2) into highly reactive ROS,[33] which in turn causes extensive cellular damage, including DNA damage, mitochondrial dysfunction, and disruption of cell membranes.[34]

According to recent research, high copper levels can accelerate the demise of cancer cells by enhancing the production of ROS and modifying related signaling pathways.[35] For example, the copper ion carrier NSC319726 activates Cu^{2+} and promotes ROS production, resulting in deoxyribonucleotide depletion and inhibition of DNA synthesis, thereby arresting cells in the G1 phase, and inducing cell death.[36] Similar to this, it has been demonstrated that the copper-binding substance disulfiram greatly increases the formation of ROS by combining with Cu^{2+} to form a complex that activates the P38 signaling pathway[37] and inhibits the NF-κB signaling pathway.[38] Through the overwhelming effect of cellular antioxidant defenses, this dual action successfully causes cancer cell death.[18] Copper ions induce cell death by increasing ROS generation and activating oxidative stress-related signaling pathways, which lead to cellular dysfunction and death.[39]

Inhibition of the Ubiquitin-Proteasome System (UPS)

The UPS is the primary pathway responsible for the degradation of nearly 80% of intracellular proteins, regulating various cellular processes such as apoptosis, signaling, and damage repair.[40] In this procedure, ubiquitin molecules are covalently conjugated to target proteins, designating them for breakdown by the proteasome.[41] Ubiquitination is crucial for cellular activities including apoptosis, signaling, and damage repair.[42]

Copper ions have been shown to interfere with the UPS through multiple mechanisms.[43] The disulfiram-Cu^{2+} complex has been shown to produce cytotoxicity in cancer cells by suppressing proteasome function, enhancing associations between

Table 1 Inducement and Outcome of Different Cell Death Pathways

Cell Death Pathway	Inducement	Outcome
Cuproptosis	Toxic accumulation of copper ions	Oxidative damage, DNA degradation, lipid peroxidation, inhibition of protease activity, and suppression of cellular proliferation.
Ferroptosis	Iron-dependent lipid peroxidation of unsaturated fatty acids	Lipid peroxidation occurs, which induces cell death.
Autophagy	Cellular stress	Degrade and recycle intracellular components to maintain cellular homeostasis.
Necrosis	Uncontrolled breakdown of cellular components	Inflammatory response
Pyroptosis	Infections	Inflammatory response
Apoptosis	Cascade of intracellular signaling pathways,	Orderly disassembly of cellular components

https://doi.org/10.2147/JIR.S498340

ubiquitin and proteins, and impeding signaling pathways upstream of the proteasome system. This complex also inhibits ATP synthase, reliant on ubiquitination, hence exacerbating proteasome failure and cellular apoptosis.[19,44] This disruption of the UPS by copper ions highlights a critical pathway through which cuproptosis can be induced.

Copper Ions Induce Cell Death by Targeting Lipoylated TCA Cycle Proteins

The disruption of TCA cycle proteins that have received lipoic acid alteration is a characteristic of cuproptosis. The regulation and proper operation of these vital metabolic enzymes depend on lipoylation, which is the covalent attachment of lipoic acid to lysine residues in TCA cycle enzymes via amide bonds.[45]

Copper ions induce proteotoxic stress and cell death by binding to these lipoylated TCA cycle proteins, leading to their aggregation and functional impairment. This results in the loss of both lipoylated proteins and Fe-S cluster proteins, which are crucial for mitochondrial function and cellular respiration.[46] FDX1 (ferredoxin 1) has been recognized as an upstream regulator of cuproptosis. Silencing of FDX1 or inhibition of enzymes associated with the lipoylation process can avert cuproptosis, highlighting the significance of these proteins in this distinct cell death mechanism. Thus, FDX1, along with other lipoylation-related enzymes such as LIPT1, LIAS, DLD, and DLAT, is pivotal to the mechanistic investigation of cuproptosis and may serve as potential biomarkers for this specific form of cell death.[8] The comprehensive understanding of the molecular mechanisms of cuproptosis establishes a basis for further investigation into its implications in disease pathogenesis and possible therapeutic applications. Figure 1 demonstrates the molecular mechanism of cuproptosis.

Abnormalities of Copper Metabolism in Liver Disease

Relationship Between Copper Accumulation and Hepatitis

Copper accumulation is intricately linked to hepatitis, particularly in copper metabolism disorders like WD.[47] A mutation in the ATP7B gene, which codes for a copper-transporting P-type ATPase, results in WD, an autosomal recessive

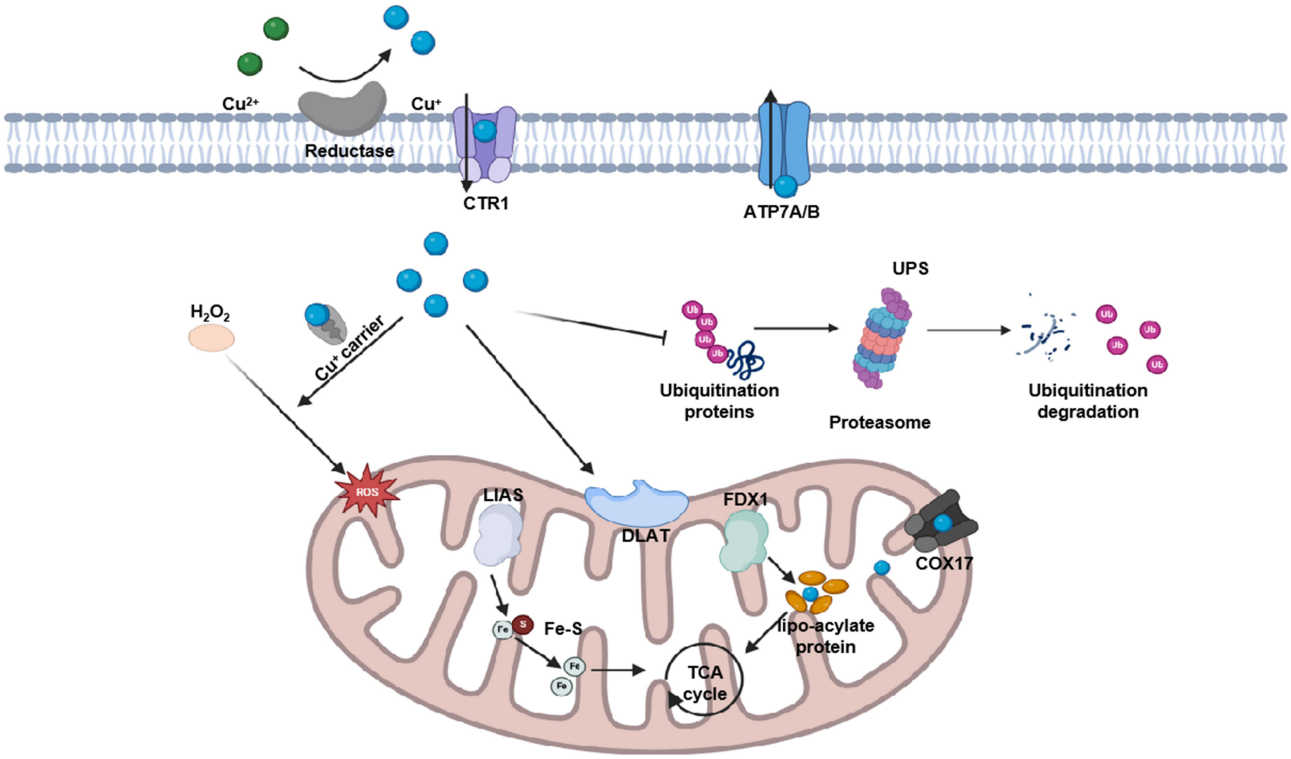

Figure 1 Cuproptosis regulates ROS, UPS and TCA.
Note: This figure is originally drawn by Figdraw platform (www.figdraw.com).

condition. In response to this mutation, the liver's capacity to remove copper is compromised, which results in an excessive buildup of copper in the liver's tissues and other organs, which eventually causes liver damage and hepatitis.[48] The excess copper induces oxidative stress, which contributes to hepatocellular injury and an inflammatory response, promoting the development and progression of hepatitis.[49] Copper accumulation exacerbates hepatic lipid metabolism, worsening the pathology of hepatitis.[50] Previous studies have indicated that oxidative stress mechanisms were the primary cause of mitochondrial dysfunction in WD patients due to high hepatic copper levels.[51] However, recent findings indicate that mitochondrial copper levels increase approximately 200-fold before hepatic copper-mediated oxidative stress occurs.[18] The inner and outer membranes of the mitochondria cross-link as a result of this substantial increase in mitochondrial copper, changing the potential of the mitochondrial membrane.[52] The observed changes correlate with reduced ATP production, inhibition of mitochondrial DNA replication, and structural alterations in mitochondria, ultimately resulting in dysfunction and apoptosis.[53] In WD patients, excessive hepatic copper disrupts nuclear receptor function and causes mitochondrial dysfunction, resulting in liver lesions.[54] Nuclear receptors play a vital role in regulating gene expression. Increased copper levels disrupt their function, leading to disturbances in lipid metabolism and contributing to liver pathology.[55] Early diagnosis and initiation of copper chelation therapy significantly improve the prognosis of WD patients and can prevent the onset of neurological symptoms.[56] Current diagnostic procedures for WD are frequently insufficient, highlighting the need for the development of novel diagnostic methodologies and biomarkers. Recent studies indicate that assessments of relative exchangeable copper and ATP7B peptide concentrations in the blood demonstrate high sensitivity and specificity for the diagnosis of WD, however further validation is required for clinical application.[57]

The pathophysiology of hepatitis may be significantly influenced by the high copper levels observed in people with chronic liver disorders. Therefore, altering copper metabolism could provide new therapeutic approaches for hepatitis treatment.[58]

MASLD and Copper Metabolism

The global rise in obesity rates has led to an increase in the number of patients with metabolic disorders.[59] The main feature of MASLD, a hepatic manifestation of metabolic syndrome (MetS), is an abnormal buildup of hepatocellular fat (≥5%) without significant alcohol intake. About 24% of people worldwide suffer from MASLD, which has a significant financial and medical impact.[60] The spectrum of liver pathology in MASLD ranges from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH), which can further progress to fibrosis, cirrhosis, and HCC in severe cases.[61] However, the pathogenesis of MASLD/MASH is still unclear.[62] According to recent research, oxidative stress plays a major role in the development of MASH9 from hepatic steatosis.[63] Oxidative stress arises from an imbalance between ROS production and antioxidant defenses, leading to cellular damage.[64] A pro-oxidant environment accelerates the course of MASLD by increasing fibrosis, inflammation, and hepatocyte death.[65] Copper ions, vital for multiple biological functions, are predominantly regulated by mitochondrial activity, where they act as cofactors for various enzymes implicated in redox balance, iron utilization, oxidative phosphorylation, and cellular proliferation.[25,66,67]

Evidence indicates that disruptions in copper homeostasis are associated with the onset of MASLD. Previous investigations indicate that hepatic and systemic copper levels are substantially reduced in animal models of MASLD, as well as in adult and pediatric patients with the condition, in comparison to healthy controls.[68] Tosco et al[69] demonstrated that copper deficiency in rats leads to hepatic steatosis by disrupting mitochondrial morphology and function and impairing fatty acid β-oxidation. Moreover, fructose intake has been demonstrated to diminish the duodenal expression of DL-1, a crucial copper transporter, therefore impairing copper absorption. Research conducted by Song et al[70] indicated that a high-fructose diet in mice led to diminished serum and hepatic copper concentrations, significantly suppressing the expression of carnitine palmitoyltransferase, a critical enzyme for fatty acid β-oxidation, while simultaneously enhancing fatty acid synthesis, ultimately resulting in hepatic steatosis and damage. Insufficient copper consumption has been linked to MASLD.[71] Diets low in copper and high in sucrose significantly upregulate genes linked to hepatic inflammation,[72] fibrogenesis, and fatty acid synthases (FAS), leading to hepatic steatosis and injury without severe obesity.[73] Decreased hepatic copper levels reduce hepatic iron export via impairing ceruloplasmin activity

and the expression of hepatic iron transporter proteins, highlighting a complicated interaction between copper and iron metabolism in MASLD.[74]

The data indicate that copper homeostasis significantly influences MASLD pathogenesis, warranting further study to elucidate its involvement in the disease's development and progression.

Insulin resistance is a key pathological driver of the metabolic abnormalities associated with MASLD, playing a pivotal role in its development.[75] Copper, an essential element for physiological processes such as glucose metabolism, plays a protective role against inflammation and oxidative damage. However, disruptions in copper homeostasis have been linked to dyslipidemia, impaired glucose tolerance, and MetS, which can potentially progress to severe hepatic steatosis and MASH.[76,77] Evidence suggests a link between elevated plasma and serum copper levels and diabetes complications, with both copper deficiency and excess contributing to oxidative stress, a precursor to insulin resistance and diabetes.[78,79] This indicates a bidirectional relationship between copper and insulin resistance in the pathogenesis of MASLD, where dietary copper imbalance—either deficiency or excess—can induce insulin resistance, hepatic steatosis, or insulin resistance-related conditions like diabetes and MetS. Chen et al[80] found that excess serum copper worsens MASLD, particularly in individuals with improved insulin resistance, highlighting a complex interaction between copper and insulin resistance. However, the small sample size in stratified analyses calls for further investigation. The emerging concept of cuproptosis offers a new avenue to explore how copper accumulation and insulin resistance drive MASLD development, underscoring the need for more comprehensive studies to unravel the connections between copper imbalance, oxidative stress, and insulin resistance in MASLD progression.

The Role of Copper Metabolism Disorders in Liver Fibrosis

Liver fibrosis is characterized by the excessive accumulation of extracellular matrix components, such as collagen, leading to tissue scarring and hardening.[81] Fibrosis results from chronic inflammation triggered by persistent infections, autoimmune reactions, and allergies.[82–84] Currently available treatments for fibrotic conditions, such as liver cirrhosis, frequently focus on the inflammatory response. However, new data indicates that the processes behind inflammation and fibrosis onset and progression are very different.[85] The substantial contribution of transition metals, particularly copper ions, to the development of fibrotic diseases, has been brought to light by recent research.[86] Copper, an essential trace element, acts as a cofactor for various enzymes. Both copper excess and deficiency can contribute to fibrotic disease progression.[87] Dysregulation of copper metabolism is strongly linked to the development of hepatic fibrosis.[88] Excess copper accumulation in the liver damages hepatocytes and activates hepatic stellate cells (HSCs), the key effectors of liver fibrosis,[89] leading to the deposition of excessive extracellular matrix and disruption of liver architecture and function.[90] The development of liver fibrosis is accelerated by copper accumulation, which is intimately related to oxidative stress and inflammatory reactions in the liver.[91] Song et al[92] demonstrated that copper deficiency worsened bile duct ligation-induced liver injury and fibrosis, due to impaired antioxidant defenses and mitochondrial dysfunction. The findings indicate that targeting copper metabolism may serve as a viable therapeutic strategy for the treatment of liver fibrosis. Some pharmacological agents have demonstrated potential in reducing hepatic fibrosis through the modulation of copper homeostasis.[93]

Potential Mechanisms of Cuproptosis in HCC

HCC is a malignant tumor with a high global mortality rate and ranks as the fourth leading cause of tumor-related deaths worldwide.[94] The prognosis for HCC is still poor despite treatment advancements, therefore finding new therapeutic targets is necessary.[95] Recent studies have suggested a potential link between dysregulated copper metabolism and HCC development.[96] Cuproptosis, a newly discovered form of cell death, is closely associated with intracellular copper accumulation.[8] Excessive copper disrupts intracellular iron metabolism, induces oxidative stress, and ultimately results in cell death.[97] In HCC cells, copper accumulation may promote tumor cell proliferation and metastasis, increasing the risk of HCC.[98] Cuproptosis-related genes (CRGs) are significantly upregulated in HCC and are associated with poor patient prognosis, suggesting their potential as therapeutic targets.[99] Clinical studies have reported elevated serum and hepatic copper levels in HCC patients,[100] which may be attributed to the downregulation of the ATP7B gene or increased cellular copper uptake.[101] Furthermore, aberrant copper metabolism has been demonstrated to impact the sensitivity of HCC cells

to chemotherapeutic drugs, thereby influencing therapy effectiveness.[102] Therefore, examining the pathways of copper metabolism in hepatocarcinogenesis should yield new insights into the molecular pathogenesis of HCC and facilitate the development of novel techniques for early diagnosis and therapy.[103]

Emerging evidence suggests that copper promotes ferroptosis in HCC, challenging the earlier view that ferroptosis is exclusively iron-dependent. Copper-binding agents like DSF-copper and elesclomol-copper disrupt mitochondrial homeostasis, leading to oxidative stress and ferroptosis in HCC cells.[104] Copper accumulation generates ROS in cancer cells, which contributes to this process. Further, copper accelerates ferroptosis by inducing the autophagic degradation of glutathione peroxidase 4 (GPX4). Copper chelators can specifically reduce sensitivity to ferroptosis without affecting other cell death pathways. Conversely, exogenous copper enhances the ubiquitination and aggregation of GPX4 by binding to its cysteine residues (C107 and C148). Tax1-binding protein 1 (TAX1BP1) then facilitates the degradation of aggregated GPX4, triggering ferroptosis in response to copper stress.[105]

Paradoxically, copper can also play an anti-ferroptosis role in HCC under certain conditions. For instance, copper stabilizes hypoxia-inducible factor-1α (HIF1α) by inhibiting prolyl-4-hydroxylase domain (PHD) enzymes in HCC cells. HIF1α, in turn, counteracts ferroptosis by upregulating genes involved in lipid metabolism. This suggests that intracellular copper may prevent ferroptosis by enhancing HIF1α expression.[106] Supporting this, copper has been shown to promote radioresistance in liver cancer cells by boosting HIF1α, which increases the transcription of CP and SLC7A11, both of which inhibit ferroptosis. Thus, copper ions act as critical signaling molecules that modulate ferroptosis sensitivity in HCC.[107] While the link between copper and ferroptosis has been recognized for decades, the precise mechanisms governing their interactions remain unclear. Further investigation into the "metallic" cross-talk between copper and other pathways in HCC is necessary to better understand their complex relationship.

Role of Copper Metabolism in Hepatitis B Virus (HBV) and HCV-Associated Hepatitis

Although the role and mechanism of copper metabolism in the regulation of viral hepatitis are still unclear, several clinical studies have suggested their relationship. Pramoolsinsap et al conducted a study on serum copper levels in young adult patients during the early phase of acute HBV infection. Their findings revealed significantly elevated serum copper levels, suggesting an alteration in copper metabolism during the acute phase of uncomplicated hepatitis.[108] Similarly, Kalkan et al investigated serum trace elements, including copper, in patients with various types of viral hepatitis (A, B, C, D, and E) compared to controls. They observed an increase in copper levels, attributing it to the body's defense mechanisms and the influence of hormone-like substances.[109] The elevated serum copper levels are likely a result of inflammatory responses triggered by the infection. Hatano et al demonstrated that liver copper content increases as liver fibrosis progresses. They concluded that elevated copper levels may contribute to the enhancement of HCV infection.[110] These results may suggest that copper accumulation in the fibrotic liver caused by chronic HCV infection may cause liver damage.

Role of Copper Metabolism in ALD

Research investigating the relationship between copper metabolism and the underlying pathophysiology of ALD remains relatively limited. Moreover, findings from these studies are often inconclusive and sometimes contradictory. For instance, Shibazaki et al reported that individuals with excessive alcohol consumption tended to exhibit significant copper deficiency, aligning with earlier evidence of disrupted copper metabolism in ALD patients.[111] Conversely, another study found that copper levels in ALD patients were either elevated or unchanged compared to healthy controls.[112] Unfortunately, most of these studies primarily focus on statistical comparisons of copper levels between patients and healthy individuals, leaving the mechanistic role of copper imbalance in the pathogenesis of ALD largely unexplored.

Clinical Importance of Copper Metabolism and Cuproptosis

Drug Development Targeting Copper Metabolism Modulation

Recent advancements in copper metabolism and its role in various diseases have led to progress in the development of therapeutic agents targeting this pathway.[113] Current research aims to develop drugs that regulate copper homeostasis to

provide therapeutic advantages for disorders linked to copper dysregulation. A study has investigated the potential of using copper ions as drug carriers to improve the targeting and bioavailability of therapeutic compounds.[114] Furthermore, there is increasing interest in developing drugs that use the cuproptosis process to modify copper uptake and excretion, thereby selectively inhibiting tumor development and making cancer cells more susceptible to traditional therapies.[115] These approaches represent promising new strategies for cancer therapy and provide hope for the effective management of other diseases linked to aberrant copper metabolism.

Clinical Studies and Therapeutic Perspectives of Abnormal Copper Metabolism

Dysregulation of copper metabolism plays a critical role in the pathogenesis of several disorders, particularly WD and related metabolic disorders.[116] Recently, there has been an increase in clinical research focused on elucidating aberrant copper metabolism and assessing different therapy approaches. A comparative study evaluating the efficiency of several treatment agents in WD patients demonstrated that specific drugs were more efficient in enhancing copper metabolism and mitigating clinical symptoms.[117] Furthermore, research has expanded to investigate the role of copper in several disorders, including diabetes, where evidence indicates that copper deficiency may contribute to the onset of diabetic complications. Therefore, regulating copper levels may alleviate these problems and enhance patient outcomes.[118] As our understanding of the molecular mechanisms regulating copper metabolism deepens, more precise and efficacious therapeutic options will probably develop for addressing disorders linked to aberrant copper metabolism.

Interventions Targeting Copper Metabolism for Liver Disease Treatment

Focusing on copper metabolism has demonstrated considerable promise in the management of liver disorders. Investigations into the processes of cuproptosis indicate that adjusting copper intake and excretion may serve as a viable therapeutic strategy for hepatic disorders resulting from copper dysregulation, including WD and NAFLD.[119] In WD patients, copper accumulation in the liver leads to hepatic damage, and treatment with copper-chelating agents has been shown to significantly improve liver function by reducing copper overload.[120] Research on NAFLD has demonstrated that dysregulation of copper metabolism is significantly linked to the onset of hepatic steatosis. Modifying dietary copper consumption has been suggested as a viable approach to enhance hepatic lipid metabolism, thereby mitigating the manifestations of fatty liver disease.[121] The findings indicate that therapeutic interventions aimed at copper metabolism may apply to other liver diseases, warranting future research into their potential use in a wider range of hepatic conditions beyond those presently examined.

Future Research Directions and Challenges

There are still many obstacles in the way of completely understanding this new type of cell death, even with the encouraging preliminary results in cuproptosis research. More research is required to fully understand the exact molecular mechanisms driving cuproptosis and how copper affects cellular metabolism and communication pathways that cause cell death.[122] Clinical trials must also thoroughly assess the safety and therapeutic potential of focusing on copper metabolism, particularly in light of different cancer types and liver diseases. For copper-modulating therapies, figuring out the ideal therapeutic window and dosage is essential to reducing possible adverse effects and maximizing effectiveness. Moreover, the interactions between copper and other trace elements, such as zinc and manganese, require comprehensive investigation to understand their collective impact on both physiological and pathological processes.[123] GSH (glutathione) serves as a central player in both ferroptosis and cuproptosis, though its roles differ significantly. In ferroptosis, GSH functions as a key antioxidant, preventing lipid peroxidation (LPO). Conversely, in cuproptosis, it acts as a copper chaperone, binding to copper ions to reduce the aggregation of fatty-acylated proteins.[22,124] Recent studies have shown that the GSH inhibitors Erastine and buthionine sulphoximine (BSO), by inhibiting GSH synthesis, induce ferroptosis and cup death.[125] These studies all suggest that there is a bridge between ferroptosis and copper death, but whether ferroptosis and copper death directly affect each other is not known.

Future research must prioritize the identification of specific biomarkers for cuproptosis across various diseases, investigate the clinical applications of therapeutic interventions, and develop personalized strategies for modulating copper metabolism to advance the field further. In conclusion, discoveries in copper research have broadened our

knowledge of the mechanisms behind disease and opened the door to innovative treatment approaches. The prospects for therapeutic uses of copper-based treatments will get more encouraging as research advances.

Data Sharing Statement

There are no data and no material associated with this manuscript.

Ethics Approval and Consent to Participate

There is no human subject, and this is a review, so there is no need for ethical approval and consent.

Consent for Publication

There are no data in this manuscript, so this is not relevant.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This review was supported by Jinhua Municipal Science and Technology Bureau Key Project (No. 2021-3-075, 2021-3-089, 2021-3-091, 2023-3-091, 2023-3-097), Jinhua Science and Technology Bureau public welfare projects (No. 2022-4-103) and Jinhua Municipal Central Hospital Science and Technology Project (No. JY2023-1-04).

Disclosure

The authors declare no competing interest.

References

1. Jasmohan SB. Alcohol, liver disease, and the gut microbiota. *Nat Rev Gastroenterol Hepatol*. 2019;16:235–46.
2. Cheng W, Cheng M, Lihong G, et al. Macrophage polarization and its role in liver disease. *Front Immunol*. 2022;12.
3. Jia X, Fei W, Nai-Kei W, et al. Global liver disease burdens and research trends: analysis from a Chinese perspective. *J Hepatol*. 2019;71:71. doi:10.1016/j.jhep.2019.03.005
4. Sumeet KA, Harshad D, John E, Patrick SK. Burden of liver diseases in the world. *J Hepatol*. 2018;69(1):70. doi:10.1016/j.jhep.2018.02.026
5. Xin-Ru W, Benjamin C. Apoptosis and Autophagy: current Understanding in Tick-Pathogen Interactions. *Front Cell Infect Microbiol*. 2022;12:784430.
6. Chun Y, Sunao L, Xinqi H, et al. Silk fibroin hydrogels could be therapeutic biomaterials for neurological diseases. *Oxid Med Cell Longev*. 2022;2022:2076680.
7. Xiuxia L, Tianhui G, Xing Z. Pyroptosis in cancer: friend or foe? *Cancers*. 2021;14:13. doi:10.3390/cancers14010013
8. Jiaming X, Yannan Y, Yibo G, Jie H. Cuproptosis: mechanisms and links with cancers. *Mol Cancer*. 2023;22:22. doi:10.1186/s12943-022-01712-8
9. Zhiwei L, Xiaojing Y, Lili G, Lijie Q, Jiabin D, Luqian Z. The link between ferroptosis and airway inflammatory diseases: a novel target for treatment. *Front Mol Biosci*. 2022;9:985571.
10. Daolin T, Xin C, Guido K. Cuproptosis: a copper-triggered modality of mitochondrial cell death. *Cell Res*. 2022;32:417–418.
11. Yiping C, Yunlei X, Jing W, Binfeng Y, Xingyu J. Click chemistry-mediated nanosensors for biochemical assays. *Theranostics*. 2016;6:969.
12. Izabela M, Katarzyna D, Mahmoud A, et al. The effect of metal-containing nanoparticles on the health, performance, and production of livestock animals and poultry. *Vet Q*. 2022;42:68–94.
13. Francesca F, Andrea G, Kevin John W. Copper microenvironments in the human body define patterns of copper adaptation in pathogenic bacteria. *PLoS Pathog*. 2022;18:e1010617.
14. Javed Y, Wasim J, Shahab A. Helicobacter pylori infection and micronutrient deficiencies. *World J Gastroenterol*. 2003;9:2137.
15. Shana JC, Farah H, William GW. A current overview of the biological and cellular effects of nanosilver. *Int J Mol Sci*. 2018;20(1):19. doi:10.3390/ijms20010019
16. Jonathon T, Ya Hui H, Stephanie M, Sharon La F, Dinse HR. Role of the P-Type ATPases, ATP7A and ATP7B in brain copper homeostasis. *Front Aging Neurosci*. 2013;5:5. doi:10.3389/fnagi.2013.00005
17. Grażyna G, Beata T, Anna F, Agata A. Copper dyshomeostasis in neurodegenerative diseases-therapeutic implications. *Int J Mol Sci*. 2020;22(1):21. doi:10.3390/ijms22010021
18. Qian X, Rui K, Daniel JK, Daolin T, Jinbao L, Xin C. Copper metabolism in cell death and autophagy. *Autophagy*. 2023;19:2175–95.

19. Valentina O. Selective targeting of cancer cells by copper ionophores: an overview. *Front Mol Biosci*. 2022;9:841814.
20. Nur Ain Asyiqin A, Yap Wing F, Nur Alia Sheh O, Wan Mohd Ebtisyam Mustaqim Mohd D, Nur Syahira Md R, Silvan S. Development of graphene quantum dots-based optical sensor for toxic metal ion detection. *Sensors*. 2019;20(1):19. doi:10.3390/s20010019
21. Atchariya C, Anil D. Wilson disease in children and young adults - state of the art. *Saudi J Gastroenterol*. 2022;28:21–31.
22. Peter T, Shannon C, Boryana P, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science*. 2022;375:1254–1261.
23. Marina S, Amedeo C, Ummi A, Massimiliano M, Alessio M. Understanding metal dynamics between cancer cells and macrophages: competition or synergism? *Front Oncol*. 2020;10:10. doi:10.3389/fonc.2020.00010
24. Tianxin L, Xudong L, Yongping S. Ferroptosis in acute leukemia. *Chin Med J*. 2023;136:886–898.
25. Qi F, Yang Y, Kaidi R, et al. Broadening horizons: the multifaceted functions of ferroptosis in kidney diseases. *Int J Biol Sci*. 2023;19:3726.
26. Zhuozhuo L, Liwei W, Yuanyuan R, et al. Arginase: shedding light on the mechanisms and opportunities in cardiovascular diseases. *Cell Death Discov*. 2022;8(1):8.
27. Xiaodan Z, Tao T, Yishu Q, Xiaojun G, Xiao Z, Xiaorong Z. Copper-mediated novel cell death pathway in tumor cells and implications for innovative cancer therapies. *Biomed Pharmacother*. 2023;168:115730.
28. Chloe S, Danish H, Rachid S. Cuproptosis: unraveling the mechanisms of copper-induced cell death and its implication in cancer therapy. *Cancers*. 2024;16:647.
29. Haoying W, Mengxiao L, Xi Z, Ya Z, Yuping W, Yongning Z. Cell death affecting the progression of gastric cancer. *Cell Death Discov*. 2022;8:8. doi:10.1038/s41420-021-00800-w
30. Liqing L, Ye Z, Xuemei T, et al. Emerging mechanisms of pyroptosis and its therapeutic strategy in cancer. *Cell Death Discov*. 2022;8:8.
31. Yohko Y, Ippei S, Tohru M. Capillaries as a therapeutic target for heart failure. *J Atheroscler Thromb*. 2022;29:971–988.
32. Viktor AT, Olga Yu S, Nikolay EP, Victoria D, George JK. Mechanistic insights of chelator complexes with essential transition metals: antioxidant/pro-oxidant activity and applications in medicine. *Int J Mol Sci*. 2022;23:1247.
33. Amira Mohammed A, Hiroshi K. Royal jelly as an intelligent anti-aging agent-a focus on cognitive aging and alzheimer's disease: a review. *Antioxidants*. 2020;9:937.
34. Bin Bin W, Kam Tong L, Ellen Ngar-Yun P. Mitochondrial-targeted therapy for doxorubicin-induced cardiotoxicity. *Int J Mol Sci*. 202223:1912.
35. Meshal A, Guo Z, Jiang A, Liang X, Tan W. Broad-spectrum theranostics and biomedical application of functionalized nanomaterials. *Polymers*. 2022;15(1):14. doi:10.3390/polym15010014
36. Shengliang Z, Lindsey C, Liz HB, Attila AS, Xiaobing T, Wafik SE-D. Advanced strategies for therapeutic targeting of wild-type and mutant p53 in cancer. *Biomolecules*. 2022;13:12. doi:10.3390/biom13010012
37. Manyu Z, Liqun W, Mengzhu W, et al. Targeting fibrosis, mechanisms, and clinical trials. *Signal Transduct Target Ther*. 2022;7:206.
38. Baisen C, Hongxiang H, Yuyu S, et al. Role of macrophage polarization in osteoarthritis (Review). *Exp Ther Med*. 2022;24:1.
39. Syed Nasir Abbas B, Kwon O-Y, Lee S-H. Dietary polyphenols as therapeutic intervention for alzheimer's disease: a mechanistic insight. *Antioxidants*. 2022;12(1):11. doi:10.3390/antiox12010011
40. Weigang L, Xun T, Xuehong Q, et al. The ubiquitin conjugating enzyme: an important ubiquitin transfer platform in ubiquitin-proteasome system. *Int J Mol Sci*. 2020;22:21.
41. Qiong L, Chanqi Y, Tian T, et al. The emerging role of ubiquitin-specific protease 20 in tumorigenesis and cancer therapeutics. *Cell Death Dis*. 2022;13:434.
42. Samantha B, Andrea G, Roberta N, Simona S, Giovanni M, Giorgia S. CDC20 in and out of mitosis: a prognostic factor and therapeutic target in hematological malignancies. *J Exp Clin Cancer Res*. 2022;41:41. doi:10.1186/s13046-022-02244-1
43. Ying L, Xiaomin X, Wenxue H, Hanlin L, Jie L, Xue L. How effective are metal nanotherapeutic platforms against bacterial infections? A comprehensive review of literature. *Int J Nanomed*. 2023;18:1109–1128.
44. Guillermin A-C, Deborah G-C, Dany D-P, et al. Emerging computational approaches for antimicrobial peptide discovery. *Antibiotics*. 2022;12:11. doi:10.3390/antibiotics12010011
45. Yuncheng W, Hao Z, Ye Z, et al. Recent advances in copper-doped titanium implants. *Materials*. 2022;15:2342.
46. Veena Wenqing X, Mohammed Zahedul Islam N, Iris xiaoxue Y, Ollie Yiru Y, Christie Ying Kei L, Chun Hung C. Application of copper nanoparticles in dentistry. *Nanomaterials*. 2022;13:12. doi:10.3390/nano13010012
47. Bruno F, Emanuel D, Miguel M-S, et al. Rheumatologic manifestations of hepatic diseases. *Ann Gastroenterol*. 2019;232.
48. Masahiro K, Ken-Ichiro T, Midori K-N. Crosstalk of copper and zinc in the pathogenesis of vascular dementia. *J Clin Biochem Nutr*. 2022;71:7.
49. Norikazu S. Diagnosis and treatment of Wilson disease in Japan. *Rinsho Shinkeigaku*. 2019;59:565.
50. Bryce B, Scott A, Ya Hui H, Ashley IB, Sharon LF. Copper and lipid metabolism: a reciprocal relationship. *Biochim Biophys Acta Gen Subj*. 2021;1865:129979.
51. Huamei L, Ran T, Lifang L, Shiqiang S. Population screening and diagnostic strategies in screening family members of Wilson's disease patients. *Ann Transl Med*. 2019;7:1.
52. Andrey VK, Johannes G. Pathogenesis of multiple organ failure: the impact of systemic damage to plasma membranes. *Front Med*. 2022;9:806462.
53. Nur Musfirah M, Luminita P, Nurliza K, Tengku Ain K. Thymoquinone in ocular neurodegeneration: modulation of pathological mechanisms via multiple pathways. *Front Cell Neurosci*. 2022;16:786926.
54. Annu A, Mohit B. The pragmatic treatment of Wilson's disease. *Mov Disord Clin Pract*. 2014;1:12–23.
55. Fernando L. Roles of estrogens, estrogen-like compounds, and endocrine disruptors in adipocytes. *Front Endocrinol*. 2022;13:921504.
56. Erica Nicola L, Claudia C, Tommaso I, Gabriele D, Paolo F, Andrea G. Practical insights into chronic management of hepatic Wilson's disease. *World J Clin Cases*. 2022;10:4334.
57. Valentina M, Janine ML. Genetics and epigenetic factors of Wilson disease. *Ann Transl Med*. 2019;7:1.
58. Isabelle M, Karl Heinz W. Biochemical markers for the diagnosis and monitoring of Wilson disease. *Clin Biochem Rev*. 2019;40:59.
59. Anna KS, Lena E, Alexandra C. Many ways to Rome: exercise, cold exposure, and diet-do they all affect bat activation and wat browning in the same manner? *Int J Mol Sci*. 202223:4759.
60. Jian-Bin Z, Meng-Ting L, Shuang-Zhe L, Yu-Qing C, Jian-Gao F, Yuan-Wen C. Therapeutic effect of prolyl endopeptidase inhibitor in high-fat diet-induced metabolic dysfunction-associated fatty liver disease. *J Clin Transl Hepatol*. 2023;11:1035.

61. Daphne V, Ralf W, Ruchi B. Therapeutic targeting of hepatic macrophages for the treatment of liver diseases. *Front Immunol*. 2019;10:10. doi:10.3389/fimmu.2019.00010
62. Tingting S, Li W, Wenjun M, et al. Nonalcoholic fatty liver disease: pathogenesis and treatment in traditional Chinese medicine and western medicine. *Evid Based Complement Alternat Med*. 2020;2020:8749564.
63. Patricia L, Marta A-P, David SS, Mayte A-L, Javier C, Marcos L-H. Innate and adaptive immunity alterations in metabolic associated fatty liver disease and its implication in COVID-19 severity. *Front Immunol*. 2021;12:651728.
64. Ming-Cheng C, Edward C. Current development, obstacle and futural direction of induced pluripotent stem cell and mesenchymal stem cell treatment in degenerative retinal disease. *Int J Mol Sci*. 2022;23:2529.
65. Irma Martha M-D, Néstor P-R, Aurora Elizabeth R-G, et al. The relationship between cancer and paraoxonase 1. *Antioxidants*. 2022;12:11.
66. Pablo C-Q, Javier G-F, Noelia S-O, José Vicente L, María Dolores M. Drosophila melanogaster models of metal-related human diseases and metal toxicity. *Int J Mol Sci*. 2017;19:18. doi:10.3390/ijms19010018
67. Lindsey C, Kelsey EH, Wafik SE-D. Immunotherapy for colorectal cancer: mechanisms and predictive biomarkers. *Cancers*. 2022;15:14. doi:10.3390/cancers15010014
68. Marie CH, Hyo Min P, Ho Yu A-Y, et al. In vivo bioluminescence imaging reveals copper deficiency in a murine model of nonalcoholic fatty liver disease. *Proc Natl Acad Sci U S A*. 2016;114:113. doi:10.1073/pnas.1618558114
69. Alessandra T, Bianca F, Rosa D, et al. Molecular bases of copper and iron deficiency-associated dyslipidemia: a microarray analysis of the rat intestinal transcriptome. *Genes Nutr*. 2009;5.
70. Ming S, Dale AS, Zhanxiang Z, et al. High fructose feeding induces copper deficiency in Sprague-Dawley rats: a novel mechanism for obesity-related fatty liver. *J Hepatol*. 2011;54:56. doi:10.1016/j.jhep.2010.06.019
71. Laura A, Cristiana P, Gino I, Clara B, Barbara B. Non-alcoholic fatty liver disease and nutritional implications: special focus on copper. *Nutrients*. 2017;9:1137.
72. Chien-Ning H, Hong-Ren Y, Julie YHC, Kay LHW, Wei-Chia L, You-Lin T. The impact of gut microbiome on maternal fructose intake-induced developmental programming of adult disease. *Nutrients*. 2022;15:14. doi:10.3390/nu15010014
73. Xing-Yu C, Cong W, Yi-Zhou H, Li-Li Z. Nonalcoholic fatty liver disease shows significant sex dimorphism. *World J Clin Cases*. 2022;10:1457.
74. Elmar A, Igor T, Heike H, et al. Copper availability contributes to iron perturbations in human nonalcoholic fatty liver disease. *Gastroenterology*. 2008;135:680–8.
75. Hironori K, Guanliang C, Yinhua N, Tsuguhito O. Nonalcoholic fatty liver disease and insulin resistance: new insights and potential new treatments. *Nutrients*. 2017;9:387.
76. Alexandra F, Elmar A, Daniel W, Katharina P. The potential role of iron and copper in pediatric obesity and nonalcoholic fatty liver disease. *Biomed Res Int*. 2015;2015:287401.
77. Ivo S, Ralf D, Julian FBM. Copper: effects of deficiency and overload. *Met Ions Life Sci*. 2014;13:359–387.
78. Pallavi D, Vikram T, Munmun C. Role of minerals and trace elements in diabetes and insulin resistance. *Nutrients*. 2020;13:12. doi:10.3390/nu13010012
79. Geir B, Maryam D, Lyudmila P, Monica Daniela D, Yuliya S, Jan A. The role of zinc and copper in insulin resistance and diabetes mellitus. *Curr Med Chem*. 2019;27:6643–57.
80. Chen C, Qi Z, Ruiyue Y, et al. Copper exposure association with prevalence of non-alcoholic fatty liver disease and insulin resistance among US adults (NHANES 2011-2014). *Ecotoxicol Environ Saf*. 2021;218.1.
81. Yosuke K, En S, Kesari KK, Kumar A. α2-antiplasmin as a potential therapeutic target for systemic sclerosis. *Life*. 2022;13(1):12. doi:10.3390/life13010012
82. Yijing R, Siyue L, Zixuan S, Qiuping L, Yingying Z, Hao W. The regulatory roles of polysaccharides and ferroptosis-related phytochemicals in liver diseases. *Nutrients*. 2022;15:14.
83. Xianli W, Xiaolin X, Yiwei X, et al. Exosome-mediated effects and applications in inflammatory diseases of the digestive system. *Eur J Med Res*. 2022;27:27. doi:10.1186/s40001-022-00652-9
84. Chantal AC, Ian W, Daniel GP, Peter JH. A spotlight on t lymphocytes in Duchenne muscular dystrophy-not just a muscle defect. *Biomedicines*. 2022;11:10. doi:10.3390/biomedicines11010010
85. Sandra Maria B, Giulia M, Vitor Fernando Bordin M, et al. Organokines in COVID-19: a systematic review. *Cells*. 2023;13:12. doi:10.3390/cells13010012
86. Ting S, Yiyuan K, Jia L, et al. Nanomaterials and hepatic disease: toxicokinetics, disease types, intrinsic mechanisms, liver susceptibility, and influencing factors. *J Nanobiotechnology*. 2021;19:19. doi:10.1186/s12951-020-00731-1
87. Erik M, Jonathan DG. Copper deficiency. *Curr Opin Gastroenterol*. 2007;2007:1.
88. Adrian F, Mirosław K, Kamil J. The role of chosen essential elements (Zn, Cu, Se, Fe, Mn) in food for special medical purposes (fsmps) dedicated to oncology patients-critical review. *State-of-the-Art Nutrients*. 2023;15:1012.
89. Joon Ho M, Chang-Myung O, Hail K. Serotonin in the regulation of systemic energy metabolism. *J Diabetes Investig*. 2022;13:1639–1645.
90. Piotr S, Anna C, Wojciech J, Tomasz L. Wilson's disease- management and long term outcomes. *Best Pract Res Clin Gastroenterol*. 2022;56:101768.
91. Mohmadshakil K, Gideon MH. Insights into the management of Wilson's disease. *Therap Adv Gastroenterol*. 2017;10:889–905.
92. Ming S, Zhanxiang Z, Theresa C, Jingwen Z, Craig JM. Copper deficiency exacerbates bile duct ligation-induced liver injury and fibrosis in rats. *J Pharmacol Exp Ther*. 2011;340:339. doi:10.1124/jpet.111.185140
93. Takashi H, Tsutomu M, Grote V. Current trends of essential trace elements in patients with chronic liver diseases. *Nutrients*. 2020;13(1):12.
94. Meng-Ping J, Wen-Xiu X, Jun-Chen H, Qi X, Dan-Dan W, Jin-Hai T. The emerging role of the interactions between circular RNAs and RNA-binding proteins in common human cancers. *J Cancer*. 2021;12:5206.
95. Maria Teresa DM, Mariamena A, Daniele C, et al. miR-221/222 as biomarkers and targets for therapeutic intervention on cancer and other diseases: a systematic review. *Mol Ther Nucleic Acids*. 2022;27:1191–224.
96. Xin H, Ronggao C, Qiang W, Xiao X. The landscape of alpha fetoprotein in hepatocellular carcinoma: where are we? *Int J Biol Sci*. 2022;18:536.

97. Zixin W, Guishuai L, Fuxue X, et al. Copper in hepatocellular carcinoma: a double-edged sword with therapeutic potentials. *Cancer Lett*. 2023;571:216348.
98. Ruo-Nan S, Kun-Hao B, Qian-Qian H, Si-Liang C, Xin H, Yu-Jun D. A novel prognostic prediction model of proptosis-related genes signature in hepatocellular carcinoma. *Front Cell Dev Biol*. 2023;11:1.
99. Dingtao H, Yichuan W, Xu S, et al. Genetic landscape and clinical significance of cuproptosis-related genes in liver hepatocellular carcinoma. *Genes Dis*. 2023;11(1):11. doi:10.1016/j.gendis.2023.01.011
100. Silvia B, Giuliana DR, Gabriele T. Current biomedical use of copper chelation therapy. *Int J Mol Sci*. 2020;22(1):21.
101. Xiangning C, Yan W, Han L, Mengjun S, Jingwu W, Yifei W. the molecular mechanisms of defective copper metabolism in diabetic cardiomyopathy. *Oxid Med Cell Longev*. 2022;2022.
102. Lanqing L, Xiaoqiang W, Haiying X, Xianqiong L, Kang X. Perspectives and mechanisms for targeting ferroptosis in the treatment of hepatocellular carcinoma. *Front Mol Biosci*. 2022;9:947208.
103. Min Z, Baibei W, Wenjie W, et al. Disulfiram: a novel repurposed drug for cancer therapy. *Chin Med J*. 2024;137:159–72.
104. Xueying R, Yanchun L, Yi Z, et al. Overcoming the compensatory elevation of NRF2 renders hepatocellular carcinoma cells more vulnerable to disulfiram/copper-induced ferroptosis. *Redox Biol*. 2021;46:102122.
105. Qian X, Ding Y, Xi C, et al. Copper-dependent autophagic degradation of GPX4 drives ferroptosis. *Autophagy*. 2023;19:1982–1996.
106. Minghua Y, Pan C, Jiao L, et al. Clockophagy is a novel selective autophagy process favoring ferroptosis. *Sci Adv*. 2019;5:eaaw2238.
107. Mi Y, Xixi W, Jinlong H, et al. COMMD10 inhibits HIF1α/CP loop to enhance ferroptosis and radiosensitivity by disrupting Cu-Fe balance in hepatocellular carcinoma. *J Hepatol*. 2022;76:1.
108. Pramoolsinsap C, Promvanit N, Kurathong S. Serum trace metal levels in patients with acute hepatitis B. *Southeast Asian J Trop Med Public Health*. 1996;27:476–480.
109. Ahmet K, Vedat B, Senel A, Ilhami C, Namik Kemal B. Trace elements in viral hepatitis. *J Trace Elem Med Biol*. 2003;16:227–30.
110. H R, E M, F H, et al. Accumulation of copper in the liver and hepatic injury in chronic hepatitis C. *J Gastroenterol Hepatol*. 2000;15:786–91.
111. Shunichi S, Shuhei U, Katsuji T, Norihide T. Copper deficiency caused by excessive alcohol consumption. *BMJ Case Rep*. 2017;2017:bcr–2017.
112. Dario R, Milan K, Zeljko R, Kristina B, Mladen P. Serum concentration of zinc, copper, manganese and magnesium in patients with liver cirrhosis. *Coll Antropol*. 2006;30:523–528.
113. Marie H, Naïma EK, Romain D, Elise L. Zebrafish as a model to study vascular elastic fibers and associated pathologies. *Int J Mol Sci*. 2022;23:2102.
114. Xidi W, Miao Z, Yu L, Zizhen S. Cope with copper: from copper linked mechanisms to copper-based clinical cancer therapies. *Cancer Lett*. 2023;561:216157.
115. Yueqin L. Copper homeostasis: emerging target for cancer treatment. *IUBMB Life*. 2020;72:1900–8.
116. Bernhard M. Review about powerful combinations of advanced and hyphenated sample introduction techniques with inductively coupled plasma-mass spectrometry (ICP-MS) for elucidating trace element species in pathologic conditions on a molecular level. *Int J Mol Sci*. 2022;23:6109.
117. Christian A-H, Tim M, Marlies LSH, Karl Heinz W, Roderick HJH, Hannah E. Comparative effectiveness of common therapies for Wilson disease: a systematic review and meta-analysis of controlled studies. *Liver Int*. 2019;39:2136–52.
118. Dongkai J, Lulu L, Wei L, Jinjie L, Xin J, Ying X. Copper metabolism and its role in diabetic complications: a review. *Pharmacol Res*. 2024;206:107264.
119. Khalid MS, Youn J, Seow WJ. The roles and pathogenesis mechanisms of a number of micronutrients in the prevention and/or treatment of chronic hepatitis, COVID-19 and type-2 diabetes mellitus. *Nutrients*. 2022;15(1):14.
120. Tingting W, Limin W, Qiuying C, et al. Copper deposition in Wilson's disease causes male fertility decline by impairing reproductive hormone release through inducing apoptosis and inhibiting ERK signal in hypothalamic-pituitary of mice. *Front Endocrinol*. 2022;13:961748.
121. Yuan L, Xiang Q, Tianzhu C, Mengyao C, Liyan W, Beihui H. Exploring the interactions between metabolic dysfunction-associated fatty liver disease and micronutrients: from molecular mechanisms to clinical applications. *Front Nutr*. 2024;11.
122. Zhibo Y, Ridong F, Hai Z. Cuproptosis and Cu: a new paradigm in cellular death and their role in non-cancerous diseases. *Apoptosis*. 2024;29:1330–60.
123. Yamei L, Ping Q, Si-Yuan S, et al. Elucidating cuproptosis in metabolic dysfunction-associated steatotic liver disease. *Biomed Pharmacother*. 2024;174:
124. Michael AB, Daniel MK, Carlo M H, et al. Cysteine depletion induces pancreatic tumor ferroptosis in mice. *Science*. 2020;368:85–90.
125. Weikai W, Kaizhong L, Xin J, et al. Ferroptosis inducers enhanced cuproptosis induced by copper ionophores in primary liver cancer. *J Exp Clin Cancer Res*. 2023;42(1):42. doi:10.1186/s13046-023-02607-2