REVIEW

Copper homeostasis and cuproptosis in health and disease

Yunuo Yang[1,2] | Jiaxuan Wu[1,2] | Lisheng Wang[3,4] | Guang Ji[1,2,*] | Yanqi Dang[1,2,*]

[1]Institute of Digestive Diseases, China-Canada Center of Research for Digestive Diseases, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China

[2]State Key Laboratory of Integration and Innovation of Classic Formula and Modern Chinese Medicine (Shanghai University of Traditional Chinese Medicine), Shanghai, China

[3]Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

[4]China-Canada Centre of Research for Digestive Diseases, University of Ottawa, Ottawa, Ontario, Canada

*Correspondence

Guang Ji, Institute of Digestive Diseases, China-Canada Center of Research for Digestive Diseases, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China.
Email: jiliver@vip.sina.com; jg@shutcm.edu.cn

Yanqi Dang, Institute of Digestive Diseases, China-Canada Center of Research for Digestive Diseases, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China.
Email: dangyanqi9022@126.com; yq_dang@shutcm.edu.cn

Abstract

Copper is a vital trace element in human physiology, essential for the synthesis of numerous crucial metabolic enzymes and facilitation of various biological processes. Regulation of copper levels within a narrow range is imperative for maintaining metabolic homeostasis. Numerous studies have demonstrated the significant roles of copper homeostasis and cuproptosis in health and disease pathogenesis. However, a comprehensive and up-to-date systematic review in this domain remains absent. This review aims to consolidate recent advancements in understanding the roles of cuproptosis and copper homeostasis in health and disease, focusing on the underlying mechanisms and potential therapeutic interventions. Dysregulation of copper homeostasis, manifesting as either copper excess or deficiency, is implicated in the etiology of various diseases. Cuproptosis, a recently identified form of cell death, is characterized by intracellular copper overload. This phenomenon mediates a diverse array of evolutionary processes in organisms, spanning from health to disease, and is implicated in genetic disorders, liver diseases, neurodegenerative disorders, and various cancers. This review provides a comprehensive summary of the pathogenic mechanisms underlying cuproptosis and copper homeostasis, along with associated targeted therapeutic agents. Furthermore, it explores future research directions with the potential to yield significant advancements in disease treatment, health management, and disease prevention.

KEYWORDS
cancer, cardiovascular disease, copper homeostasis, cuproptosis, hereditary disease, liver disease, neurodegenerative disease

1 | INTRODUCTION

Copper is a vital trace element within the human body, with the current recommended daily intake for adults ranging from 0.8 to 2.4 mg/day.[1] The majority of copper is stored in muscle and bone (50–70%), with smaller amounts found in blood (5-10%) and the liver containing the highest concentration among internal organs (approximately 20%). Copper plays a crucial role in various physiological processes such as energy metabolism, antioxidant defense, blood clotting, neurotransmitter synthesis, and iron metabolism.[1,2] Elevated concentrations of copper within the body can impede cellular homeostasis by perturbing redox equilibrium, resulting in cellular apoptosis.[3] Conversely, insufficient levels of copper can hinder growth and immune function, potentially leading to the onset of various pathologies.

There has been a strong correlation between copper homeostasis and cuproptosis, as well as the progression of human health and disease. These processes are implicated in various evolutionary transitions from health to disease in organisms. This review systematically compiles and synthesizes the latest research findings on copper homeostasis and cuproptosis in the context of health and disease, examines their potential applications, and offers perspectives for future research directions.

Copper, being an essential trace element, plays a significant role in human health and disease. In the scientific community, copper homeostasis and cuproptosis have recently gained increasing attention due to the emergence of cuproptosis. The aim of this review is to provide an overview of copper homeostasis and cuproptosis, focusing on the mechanisms involved in maintaining copper homeostasis, particularly in the context of cuproptosis, including hereditary diseases, liver diseases, neurodegenerative diseases, cancers, and cardiovascular diseases. In addition to examining pertinent pharmaceuticals derived from these targets, this review will provide a comprehensive overview of the impact of copper homeostasis and cuproptosis on human health and disease, encompassing the underlying mechanisms and potential therapeutic approaches.

2 | COPPER HOMEOSTASIS AND CUPROPTOSIS IN HEALTH

2.1 | Copper homeostasis

2.1.1 | Historical background

As a transition metal with inherent redox capabilities, copper plays a critical role in biological processes, including the synthesis and activation of essential metabolic enzymes such as lysyl oxidase (LOX), cytochrome *c* oxidase (COX), and intracellular copper-zinc superoxide dismutase (SOD1).[4,5] Copper, as an essential enzyme cofactor, plays a vital role in antioxidant processes, biological synthesis, bone formation, energy metabolism, and iron metabolism, among other physiological functions.[6,7]

Studies have shown that dysregulation of copper homeostasis induces oxidative stress. Activation of Fenton-like reactions by high copper concentration facilitates the formation of reactive oxygen species (ROS), consequently impairing cell morphology and enhancing lipid peroxidation. Furthermore, due to disruption of the ubiquitin–proteasome system, copper ions inhibit cellular protease activity and cell proliferation. Excessive copper ions can also trigger cuproptosis by promoting the aberrant oligomerization of lipoylated proteins. Additionally, copper is crucial for the maintenance of normal physiological cardiovascular function, and the copper transport protein 1 (CTR1) promotes the internalization through sulfinylation, thereby enhancing angiogenesis.[8]

Iron plays a crucial role in maintaining the body's physiological functions. Research suggests that copper can impact iron metabolism, particularly through its involvement in LOX and ceruloplasmin functions.[10,9] Deficiency in ceruloplasmin can disrupt iron metabolism and transportation, leading to abnormal iron accumulation in the body. Moreover, copper deficiency can manifest as hypochromic microcytic anemia, akin to iron deficiency.[11–13] Furthermore, copper has been discovered to promote iron homeostasis within the body.[14] Copper facilitates the release of iron from storage by promoting the biosynthesis of ceruloplasmin, an enzyme responsible for oxidizing iron in circulation.[9] Copper is redistributed to tissues and organs involved in regulating iron homeostasis, including the intestines, liver, and blood, during episodes of iron deficiency.[9] Arredondo et al.[15] discovered that copper inhibits iron uptake in human colon adenocarcinoma cells, while conversely, iron inhibits copper uptake. The mechanism of iron uptake by the brain may undergo alterations in the presence of excess copper. Furthermore, a study has demonstrated that copper loading led to a reduction in iron uptake in relevant organs of rats.[16] An animal experiment indicated that dietary copper deficiency resulted in iron-deficiency anemia, potentially linked to decreased iron absorption and retention.[17] Excessive copper overload suppresses the activity of divalent metal transporter 1, consequently impacting the intestine's absorption of iron.[9,18,19]

2.1.2 | Metabolism of copper

Copper is primarily absorbed into the human body through food intake. Foods rich in copper include oysters,

scallops, pork liver, walnuts, oats, and buckwheat. In the small intestine, copper absorption primarily occurs, where Cu^{2+} is converted to Cu^{1+} by six-transmembrane epithelial antigen of the prostate (STEAP, a metalloreductase). Afterward, it is taken up by the CTR1 in the epithelial cells of the small intestine. Copper then enters the bloodstream via the copper ion transport ATPase alpha peptide (ATP7A), which facilitates its distribution to all tissues throughout the body.[1] Copper is mainly stored in the liver. The excess copper is mainly excreted into the intestines through bile and then eliminated in feces.[20–22]

Under normal physiological conditions, the content of copper within cells is maintained at a narrow range under the influence of a sophisticated network of proteins. This network comprises copper enzymes, copper chaperones, and membrane transporters, working in concert to coordinate the regulation of copper influx, efflux, and intracellular utilization.[23] Copper enters cells and is shuttled to subcellular compartments with the aid of STEAP and CTR1. Within mitochondria, essential metabolic enzymes such as COX and SOD1 serve as critical components, pivotal for scavenging free radicals and safeguarding against oxidative damage.[24] Copper serves as a vital cofactor for COX, essential for maintaining normal mitochondrial respiration.[25] Copper initially binds to COX copper chaperone 17 (COX17), from where it is subsequently transported to COX through the action of COX11 and COX synthesis 1.[26] On the other hand, the maturation of SOD1 relies on the insertion of copper ions,[27] facilitated by copper superoxide dismutase chaperonin.[28] Within the nucleus, copper participates in gene expression by interacting with transcription factors.[20] Moreover, facilitated by the antioxidant 1 copper chaperone, copper is translocated to ATP7A and ATPase beta peptide (ATP7B) within the trans-Golgi network, eventually reaching the lumen.[29]

2.1.3 | Introduction of copper oxidase and its physiological function

LOX enzymes are part of the secreted LOX family and are extracellular copper-dependent enzymes.[30] Studies indicate that various organs, including the heart, kidneys, lungs, liver, and lung, have been found to have profibrotic effects when these enzymes cross-link collagen and elastin.[31] Peptides derived from the N-terminus of LOX may exhibit tumor-suppressive properties.[32] Furthermore, this enzyme family is implicated in cell proliferation, differentiation, and the development of cardiovascular diseases.[33]

COX serves as the terminal enzyme in the electron transport chain, functioning as both an oxygen acceptor and a rate-limiting enzyme in mitochondrial respiration. It cat-

alyzes the oxidation of cytochrome *c*, playing a crucial role in ATP generation.[34,35] Copper acts as a cofactor for the COX enzyme, being translocated to the mitochondria and subsequently inserted into the subunits COX1 and COX2, which are essential for maintaining the activity of enzyme.[36]

By disproportionating superoxide anion radicals, SOD1 scavenges ROS by promoting oxygen and hydrogen peroxide production, thus participating in the dynamic balance between oxygen and hydrogen peroxide.[37] Moreover, research has demonstrated that SOD1 functions as a transcription factor in the regulation of gene expression and plays a role in metabolic pathways.[38] The involvement of SOD1 in the pathogenesis of various diseases, such as familial amyotrophic lateral sclerosis (ALS), has been well documented.[38] Additionally, studies have suggested a potential link between SOD1 and tumorigenesis, as elevated SOD1 levels are associated with poorer prognosis for various cancer types, such as breast and lung cancers.[39] Furthermore, SOD1 has been implicated in the processes of aging and oxidative stress.[37]

2.2 | Cuproptosis

2.2.1 | Core components and regulatory mechanisms of cuproptosis

Although copper is necessary for organism growth, it is important to consider the potential risks of copper overdose.[29] In 2022, Tsvetkov et al.[23] first described cuproptosis, a process in which intracellular copper accumulation leads to the binding of copper to thiooctylated components of the tricarboxylic acid cycle. This results in abnormal aggregation of lipoylated protein, disrupting mitochondrial respiration-associated Fe−S cluster proteins, and ultimately leading to a proteotoxic stress response that results in cell death.[23] Ferredoxin 1 (FDX1) functions as an upstream regulator of proteolipid acylation, with immunohistochemical staining revealing a strong correlation between FDX1 expression and lipoylated protein levels. Both FDX1 and proteolipid acylation play crucial roles in the regulation of cuproptosis.

The onset of cuproptosis is typically associated with distinct cellular morphological alterations. Animal studies have demonstrated that copper treatment induces mitochondrial contraction, chromatin rupture, endoplasmic reticulum damage, and cellular membrane rupture in zebrafish embryonic retinal cells.[40]

Mitochondria play a crucial role in the process of cuproptosis, which involves aerobic respiration and energy production in cells.[41] Tsvetkov and colleagues[42] observed
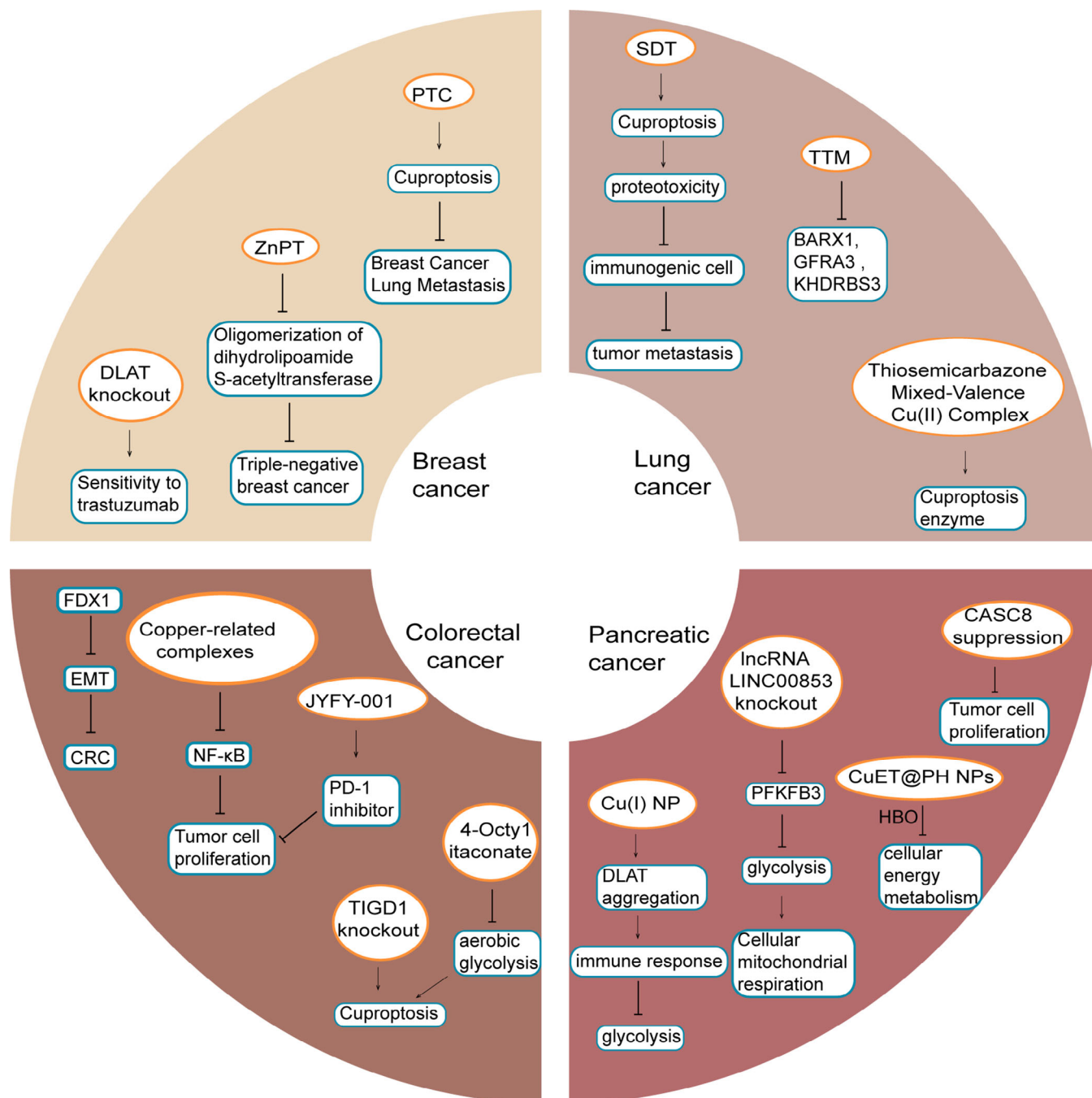
FIGURE 1 Copper homeostasis and cuproptosis in cancer. In colorectal cancer (CRC), copper-related complexes have been shown to impede tumor cell proliferation by inhibiting the NF-κB pathway in colorectal cancer cell lines. Additionally, ferredoxin 1 (FDX1) has been found to hinder the growth and advancement of CRC by suppressing epithelial–mesenchymal transition (EMT). Furthermore, JYFY-001 has been demonstrated to enhance the antitumor effects of programmed cell death protein 1 (PD-1) inhibitors. Moreover, 4-octyl itaconate inhibits aerobic glycolysis by specifically targeting glyceraldehyde-3-phosphate dehydrogenase, thereby facilitating cuproptosis. Knockdown of tigger transposable element derived 1 (TIGD1) has been observed to potentially enhance cuproptosis in CRC cells. In breast cancer, a platelet vesicle (PV)-coated cuprous oxide nanoparticle (Cu₂O)/TBP-2 cuproptosis sensitization system (PTC) has been shown to inhibit metastasis by targeting and inducing cuproptosis. Additionally, knockdown of DLAT has been found to increase breast cancer sensitivity to trastuzumab. Zinc pyrithione hinders the advancement of triple-negative breast cancer by stimulating the oligomerization of dihydrolipoamide S-acetyltransferase. In the context of lung cancer, thiotetramide has the potential to counteract the increased expression of BarH-like homeobox 1 (BARX1), GDNF family receptor alpha 3 (GFRA3), and KH domain containing RNA binding, signal transduction associated 2 (KHDRBS2). Elevated levels of copper in vivo lead to cuproptosis, a process that is intensified by SDT resulting in proteotoxicity, which leads to the destruction of immunogenic cells and the suppression of tumor metastasis. Thiosemicarbonyl mixed-valent copper(II) complexes have the ability to eliminate lung cancer cells by activating various pathways, including cuproptosis enzymes. In pancreatic cancer, the inhibition of

that cells with a greater reliance on mitochondrial respiration exhibit heightened sensitivity to copper ion carriers compared with cells primarily undergoing glycolysis, such as embryonic retinal cells. This association has significant implications for understanding the mechanisms of cell death, particularly in the context of cuproptosis. Excessive copper accumulation has been shown to disrupt mitochondrial structure and function by enhancing membrane permeability and compromising membrane potential.[43] Furthermore, mitochondrial glutathione has been demonstrated to inhibit cuproptosis by reducing thiooctylation and promoting dihydrolipoamide S-acetyltransferase (DLAT) oligomerization.[44]

It is essential to regulate copper levels within a precise range in the body. When there is a dysregulation in the metabolic homeostasis of copper leading to abnormal levels of copper, it can result in the development of associated diseases.

3 | COPPER HOMEOSTASIS AND CUPROPTOSIS IN CANCER

Cuproptosis has been linked to tumor development, prompting increased research interest in the relationship between cuproptosis and cancer. Analysis of pan-cancer multiomics and single-cell sequencing data has revealed significant expression of cuproptosis-associated genes across various tumor types, with cyclin dependent kinase inhibitor 2A exhibiting the highest mutation frequency (49%) among all tumors. The study found a negative correlation between cuproptosis scores and tumor microenvironment scores, but a positive correlation with PD-L1 expression. Additionally, cuproptosis was associated with poor tumor prognosis[45] (Figure 1).

3.1 | Colorectal cancer

Colorectal cancer (CRC), a prevalent malignancy in the gastrointestinal tract, is influenced by various unfavorable risk factors including obesity, population aging, smoking, and lack of physical activity.[46] In terms of global incidence and mortality, CRC is the third most prevalent cancer worldwide,[47] constantly threatening human public health safety.

Colorectoscopy remains the preferred method for diagnosing CRC, yet its semi-invasive nature often leads to low patient compliance and limited clinical implementation, resulting in delayed diagnoses in many cases. Recent advancements in molecular biology technology have expanded the options for detecting and treating CRC. Copper-mediated cuproptosis and its associated proteins serve as valuable indicators for the diagnosis and prognosis of CRC.

Studies have recently demonstrated that serum copper levels are associated with CRC. One study revealed that blood copper levels exceeding 930 μg/L were linked to a heightened risk of developing CRC, suggesting that blood copper levels could serve as a potential indicator for early detection of the disease.[48] Conversely, an examination of 4663 participants found no statistically significant correlation between serum copper levels and CRC.[49] Furthermore, a meta-analysis encompassing 26 studies indicated that individuals with CRC exhibited higher average copper levels compared with controls, although this disparity did not reach statistical significance.[50]

Copper-related complexes inhibit tumor cell proliferation by inhibiting the nuclear factor kappa-B (NF-κB) pathway.[51] Shao et al.[52] examined the association between the expression of cuproptosis-related molecules and CRC, finding that the levels of cuproptosis-related molecules were linked to tumor node metastasis (TNM) stage, pathological characteristics, tumor immune microenvironment, and drug resistance in CRC patients. Specifically, high DLAT expression in CRC patients was associated with chemotherapy and immunotherapy resistance, but also correlated with improved prognosis and could serve as a potential biomarker for CRC.[52] Research findings suggest that DLAT is linked to immune-related pathways, the tumor microenvironment, and various immune cell infiltrations.[53] Additionally, Li et al.[54] conducted an analysis on cuproptosis-related characteristics in CRC and established a risk score prediction model consisting of seven copper-related genes (CRGs, including dipeptidyl peptidase 7, G protein-coupled receptor associated sorting protein 1, unc-5 netrin receptor C, RAB3B, the member of RAS oncogene family, protocadherin 9, solute carrier family 18 member A2, cerebellar degeneration related protein 2 like). Patients with a high risk score exhibited a shorter overall survival and lower complete

cancer susceptibility candidate 8 (CASC8) impacts the proliferation of pancreatic cancer cells. The Cu(I) nanoparticles induce the aggregation of dihydrolipoamide S-acetyltransferase (DLAT), resulting in cuproptosis, while also enhancing immune responses and suppressing tumor growth. Copper nanodrug dopamine and hydroxyethyl starch-stabilized copper diethyldithiocarbamate nanoparticles (CuET@PH NPs) effectively suppress energy metabolism in pancreatic ductal adenocarcinoma stem cells under hyperbaric oxygen conditions. Knockdown of the cuproptosis-associated long noncoding RNA LINC00853 inhibits glycolysis and enhances cellular mitochondrial respiration by reducing the expression of the glycolytic enzyme glycolytic kinase 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3).

remission rate.[54] Furthermore, Gong et al.[55] developed a predictive model for CRC focusing on cuproptosis and disulfidptosis, which demonstrated strong predictive ability. Zhu et al.[56] utilized a principal component analysis algorithm to develop a cuproptosis score, which showed that patients with a lower cuproptosis score demonstrated increased survival, immune cell activation, and tumor purity. Additionally, FDX1 was found to have a negative correlation with tumor invasion in colon adenocarcinoma according to database analysis.[57] Furthermore, FDX1 was shown to impede the growth and progression of CRC by inhibiting epithelial-mesenchymal transition.[58] Wu et al.[59] further confirmed that knockdown of tigger transposable element derived 1 may enhance cuproptosis in CRC cells. 4-Octyl itaconate has been shown to inhibit aerobic glycolysis by targeting glyceraldehyde-3-phosphate dehydrogenase, leading to the promotion of cuproptosis and its potential role as an anti-CRC agent.[60] Additionally, the upregulation of the cuproptosis-related gene miR-653 in CRC tissues has been linked to increased cell proliferation and decreased apoptosis through negative regulation of dihydrolipoamide dehydrogenase (DLD) expression.[61] Furthermore, Shi et al.[62] have introduced a novel copper chelator, JYFY-001, which demonstrates efficacy in inhibiting tumor cell proliferation, inducing apoptosis, and enhancing the antitumor effects of programmed cell death protein 1 (PD-1) inhibitors, while also exhibiting a favorable safety profile.

3.2 | Breast cancer

Breast cancer is a prevalent malignancy affecting the epithelium of the breast or ductal epithelium. It can be categorized into three subtypes: Hormone positive, human epidermal growth factorreceptor 2(HER2)MYC proto-oncogene, bHLH transcription factor positive, and triple-negative breast cancer.[63] The global incidence of breast cancer is one in 20 individuals, with a higher prevalence of one in eight individuals in high-income countries.[64] Risk assessment models have been developed to estimate an individual's likelihood of developing breast cancer, and advancements in technology have enhanced the accuracy of these predictive models. Sha et al.[65] developed a nomogram to predict survival probability in triple-negative breast cancer through analysis of databases. Patients with low CRG-scores exhibited elevated tumor mutational load and immune activation, correlating with favorable survival outcomes.[65]

Cuproptosis-associated long noncoding RNAs (lncRNAs) may play a role in breast cancer regulation. Researchers utilize six cuproptosis-associated lncRNAs to construct a comprehensive model for prognostic prediction and therapy sensitivity assessment in breast cancer

patients, aiding in clinical treatment decision-making.[66] Immunohistochemical analysis of breast cancer surgical specimens, coupled with bioinformatics analysis, demonstrated a significant correlation between MYC proto-oncogene, bHLH transcription factor(c-Myc)AIE photosensitizer expression and the stemness phenotype, as well as copper-induced cytotoxicity, in breast cancer tissues. Furthermore, the presence of protumor cuproptosis biomarkers was found to be associated with tumor cell stemness.[67] Utilizing a database, researchers identified solute carrier family 31 member 1 (SLC31A1) as a potential cuproptosis-related gene that is upregulated in breast cancer and is linked to poor patient prognosis.[68]

Through extensive investigation of cuproptosis-related targets, a range of novel drugs have been developed. Ning et al.[69] developed a platelet vesicle-coated cuprous oxide nanoparticle/AIE photosensitizer(TBP-2)lipoylated protein cuproptosis system that effectively hinders lung metastasis of breast cancer by selectively targeting and inducing cuproptosis in tumor cells, while concurrently enhancing central memory T cells in peripheral blood and preventing tumor reinvasion. Additionally, the tumor suppressor p53 plays a crucial role in regulating the formation of iron-sulfur clusters and the copper chelator glutathione, thereby modulating intracellular cuproptosis levels in a bidirectional manner.[70] Subsequent investigations have identified LINC01614 as a potential upstream regulator of SLC31A1.[71] A comprehensive analysis across multiple cancer types indicates that diminished expression of copper-related genes ATP7B and DLAT is linked to enhanced immune response activation, improved patient survival rates, and heightened responsiveness to chemotherapy.[72] Specifically, DLAT has been identified as a factor contributing to resistance against targeted therapeutic interventions. Suppression of DLAT expression has been shown to heighten the sensitivity of breast cancer cells to trastuzumab, with the gene playing a role in the regulation of organelle fission, chromosome segregation, nuclear division, hormone-mediated signaling pathways, and chromosome condensation.[73] Zinc pyrithione has been shown to impede the advancement of triple-negative breast cancer by perturbing copper homeostasis through the facilitation of DLAT oligomerization, a key indicator of cuproptosis.[74]

3.3 | Lung cancer

Lung cancer, arising from the tracheal, bronchial mucosal, or glandular tissues, is the most prevalent malignant neoplasm affecting the lung. Histopathologically, it is predominantly categorized into non-small cell carcinoma (comprising adenocarcinoma and squamous carcinoma)

and small cell carcinoma.[75] Based on statistical data, lung cancer constitutes 11.6% of all cancer cases and is the predominant form of cancer, leading to the highest mortality rates attributed to cancer. Factors such as pollution and smoking are frequently cited as common contributors to the development of lung cancer.[76] Approximately 2 million new cases of lung cancer are reported annually, resulting in a mortality range of 10−76 million deaths.[77]

Zhang et al.[78] utilized database analysis to identify five oncogenic driver genes associated with lung cancer, including CD79b molecule, phosphatidylethanolamine binding protein 1, protein tyrosine kinase 2 beta, syntaxin binding protein 1, and zinc finger protein 671, and subsequently developed a proportional hazard regression model. The study results indicated a notable decrease in survival rate among the high-risk group.[78] Chen et al.[79] developed a gene signature for lung adenocarcinoma (LUAD) and observed elevated expression of BarH-like homeobox 1, GDNF family receptor alpha 3, and KH domain containing, RNA binding, signal transduction associated 2 in an in vitro cuproptosis model. Additionally, they found that the copper chelator tetrathiomolybdate ammonium could reverse this up-regulation.[79] Xie et al.[80] created a gene score to predict overall survival, revealing that patients with low CRG scores had increased tumor mutational load, enhanced immunoreactivity, and a greater likelihood of survival. Yang et al.[81] developed a prognostic signature utilizing eight cuproptosis-associated genes to forecast overall survival in patients with LUAD, and subsequently illustrated the impact of secreted phosphoprotein 1 on the proliferation, migration, and stemness of LUAD cells. Wang et al.[82] devised a nomogram incorporating ten cuproptosis-associated lncRNAs. Varied risk categories exhibit distinct sensitivities to conventional therapeutic modalities for lung cancer, thereby offering valuable guidance for the selection of diverse treatment strategies in clinical practice.[82] Ma et al.[83] identified nine lncRNAs associated with immune checkpoints in LUAD. The key cuproptosis gene pyruvate dehydrogenase E1 component subunit alpha (PDHA1), which is crucial for cuproptosis, plays a role in altering glucose metabolism. High levels of PDHA1 in LUAD patients are linked to a negative prognosis.[84] Researchers developed prognostic scores for non-small cell lung cancer using CRGs, revealing that the high-risk group with high prognostic scores had worse outcomes, increased tumor mutational load, and reduced immune infiltration compared with those with high cuproptosis scores.[85] The study revealed a correlation between cuproptosis and ferroptosis in LUAD, with genomic analyses illustrating the interplay between copper and iron oxidase regulators. This information was utilized to develop the Cu-Fe score, a tool for predicting treatment outcomes with various therapies.[74] Additionally, sorafenib and erastin, known inducers of ferroptosis, were found to inhibit FDX1 degradation, enhance the aggregation of copper-dependent lipoylated proteins, and stimulate cuproptosis in primary hepatocellular carcinoma (HCC) cells.[86] In vivo and in vitro studies have demonstrated that thiosemicarbonyl mixed-valent copper (II) complexes have the ability to induce cell death in lung cancer cells through the activation of multiple pathways, including cuproptosis enzymes.[87] More recently, researchers have developed copper-substituted zinc–aluminum ternary layered double hydroxide nanosheets for the combined treatment of lung cancer metastasis using acoustic-dynamic therapy (SDT). This treatment involves the use of excess copper in vivo to trigger cuproptosis, which is further enhanced by SDT to induce proteotoxicity, leading to the elimination of immunogenic cells and the inhibition of tumor metastasis.[88]

3.4 | Pancreatic cancer

Pancreatic cancer, a malignancy arising from the epithelial and follicular cells of the pancreatic ducts, ranks as the seventh most common cause of cancer-related mortality globally.[89] The incidence of pancreatic cancer has increased by over twofold in the past quarter-century due to demographic shifts and lifestyle modifications. Risk factors for the disease encompass advanced age, tobacco use, obesity, diabetes, alcohol consumption, and chronic pancreatic injury.[90] Timely detection of tumors is imperative for a favorable prognosis; however, over 80% of patients are in advanced stages of the disease, resulting in a 5-year survival rate and prognosis that is notably poor.[89] Consequently, it is crucial to promptly evaluate patient prognosis and determine suitable treatment modalities. Recent research has implicated copper in the pathogenesis of pancreatic cancer, suggesting that cuproptosis may serve as a valuable tool for prognostic assessment in patients. The identification of cuproptosis-related characteristics utilizing five lncRNAs has the potential to serve as a prognostic tool for individuals with pancreatic cancer. Among these lncRNAs, cancer susceptibility 8 exhibits heightened expression levels and influences the immune microenvironment of tumors. Subsequent investigations have revealed that inhibiting cancer susceptibility candidate 8 impacts the proliferation, apoptosis, migration, and chemokine expression in pancreatic cancer cells.[91] Sun et al.[92] developed a prognostic signature for pancreatic adenocarcinoma comprising four immune-related genes to forecast patient outcomes and immune profiles, thereby enhancing the ability to tailor personalized combination therapies centered on immunotherapy. In the context of pancreatic cancer, the down-regulation of cuproptosis-

associated lncRNA LINC00853 hinders glycolysis and enhances cellular mitochondrial respiration through the reduction of glycolytic kinase.[93] Furthermore, DLAT expression is correlated with immune cell infiltration, making it a potentially valuable prognostic and immunological biomarker for pancreatic adenocarcinoma. Some researchers have constructed risk assessment models with high predictive accuracy based on DLAT expression levels.[94]

Recently, researchers have introduced several innovative drugs aimed at targeting cuproptosis. Yu et al. have developed cascade nanosystems that, when administered to tumor cells, release copper ions to induce cuproptosis, thereby supporting chemokinetic therapy to augment the efficacy of combination treatments.[95] Copper complex nanoparticles (Cu(I) NP) have also been shown to trigger the release of copper complexes, disrupt mitochondrial function, facilitate the aggregation of DLAT, ultimately resulting in cuproptosis, as well as eliciting immune responses and impeding tumor progression.[96] An investigator recently published a study detailing the use of an acoustic wave-triggered cascade lactate depletion strategy with a semiconductor polymer nanoreactor for cuproptosis immunotherapy in pancreatic cancer. This approach utilizes Cu^+ to activate antitumor immune effects through cuproptosis, resulting in the death of immunogenic cells.[97] Additionally, copper nanodrug dopamine and hydroxyethyl starch-stabilized copper diethyldithiocarbamate nanoparticles (CuET@PH NPs) were developed based on cuproptosis, effectively inhibiting energy metabolism in pancreatic ductal adenocarcinoma stem cells under hyperbaric oxygen conditions.[98] Numerous studies have elucidated the intricate relationship between copper homeostasis and various types of cancers. The correlation between copper levels, particularly cuproptosis, and diverse cancers has been established, with current research efforts concentrated on investigating the association between CRGs and cancer progression, prognosis, and drug resistance through data analysis and the development of predictive models. However, there remains a lack of conclusive biological evidence regarding the specific mechanisms through which these genes contribute to disease development, necessitating further exploration.

4 | COPPER HOMEOSTASIS AND CUPROPTOSIS IN NEURODEGENERATIVE DISEASE

There are several neurodegenerative diseases associated with copper homeostasis and cuproptosis, such as Alzheimer's disease (AD), ALS, and Huntington's chorea (HD)[99] (Figure 2).

4.1 | Alzheimer's disease

AD, a degenerative disorder of the central nervous system marked by progressive cognitive decline and behavioral impairment, is the most prevalent form of dementia.[100] The prevalence of AD is projected to increase twofold in Europe and threefold globally by the middle of the century.[101] Despite the lack of consensus on the etiology of AD, several hypotheses have been proposed to elucidate its pathogenesis. The initial theory posited in the study is the amyloid cascade hypothesis, which suggests that the production of β-amyloid (Aβ) through the hydrolysis of amyloid precursor protein by β- and γ-secretase proteins leads to an increased ratio of hydrophobic Aβ1-42/43. This accumulation of Aβ in the brain results in the formation of age spots and subsequently triggers inflammatory reactions, neurotoxicity, and a series of pathological processes. These processes further enhance the deposition of Aβ, creating a cascade of amplified responses.[102] The subsequent hypothesis involves the abnormal phosphorylation of tau proteins. The tau protein, a microtubule-associated protein, plays a crucial role in maintaining cytoskeletal stability. Abnormal phosphorylation of tau protein results in the formation of double-stranded helical filaments and neuronal fiber tangles, ultimately leading to microtubule collapse and neuronal death. Furthermore, various theories such as the cholinergic theory, oxidative stress hypothesis, and neuroinflammation hypothesis have been proposed to explain the pathogenesis of AD.[103,104]

Copper metabolism disorders have also been linked to the progression of AD. A bibliometric analysis of 73 studies revealed alterations in copper, iron, zinc, and selenium levels in AD patients.[105] A meta-analysis indicates a reduction in copper levels in the brains of patients with AD and an elevation in serum/plasma levels, correlating with an augmented risk of AD.[106] Recent research has identified disruptions in metal ion homeostasis, particularly copper, in vulnerable brain regions of individuals with AD, facilitating the aggregation of Aβ and its interaction with tau. The excessive buildup of copper additionally fosters cuproptosis.[103] The direct interaction between the copper-binding domain of amyloid precursor protein and various amino acid residues of Aβ may contribute to the pathogenesis of AD.[107] A study involving 20 patients diagnosed with AD demonstrated that the copper chelator Clioquinol effectively decreased levels of tau protein and growth-associated protein by day 21.[108]

4.2 | Amyotrophic lateral sclerosis

ALS is a chronic and progressive neurological disorder characterized by symptoms of skeletal muscle weakness,
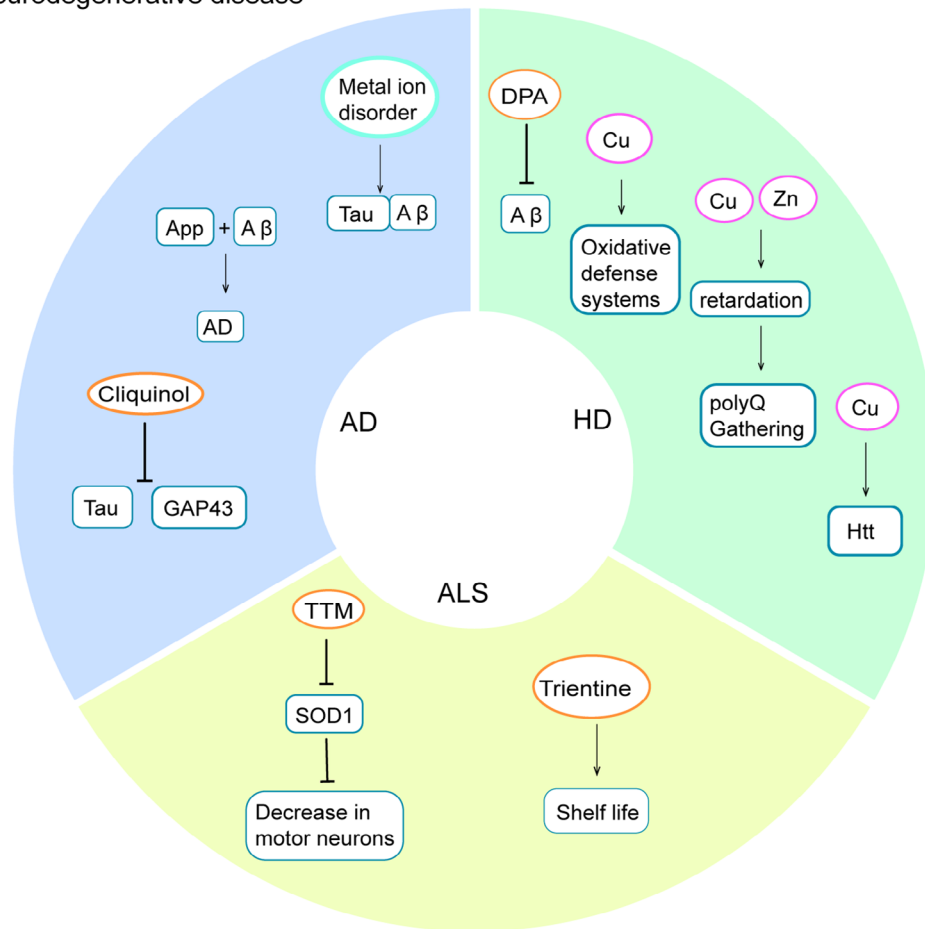
FIGURE 2 Copper homeostasis and cuproptosis in neurodegenerative disease. In Alzheimer's disease (AD), disruption of metal ion homeostasis contributes to the accumulation of β-amyloid (Aβ) and tau proteins. The interaction between the copper-binding domain of amyloid precursor protein (APP) and specific amino acid residues of Aβ may play a role in the pathogenesis of AD. Treatment with the copper chelator clioquinol has been shown to decrease levels of Tau protein and growth-associated protein. In amyotrophic lateral sclerosis (ALS), treatment with tetrathiomolybdate ammonium (TTM) has been found to reduce the formation of superoxide dismutase (SOD1) aggregates and mitigate the loss of motor neurons. Administering trientine prior to disease onset significantly extends the lifespan of ALS mice. In Huntington's disease (HD), D-penicillamine (DPA) has been shown to decrease the aggregation of β-amyloid in a drosophila model of HD. Copper has been found to enhance the formation of thioflavin S-positive β-amyloid structures within the huntingtin (HTT) protein aggregates and to activate the antioxidant defense system. Prolonged exposure to copper, zinc, and their combinations has been shown to lead to the inhibition and aggregation of polyglutamine (polyQ) in both muscle and neuronal tissues.

atrophy, fasciculations, and medullary paralysis, ultimately leading to respiratory failure and death within approximately three years.[109] The exact cause of ALS remains uncertain, with potential factors including genetic predisposition, abnormal immune responses, lifestyle choices, and metabolic dysregulation. Despite significant research efforts, the current diagnosis and treatment of ALS continue to be less than ideal.[110] Recent advancements in molecular biology have highlighted the importance of targeted therapies for maintaining copper homeostasis.

It has been proposed that alterations in the SOD1 gene are crucial in the onset of ALS, as abnormalities in this gene may cause mitochondrial issues, harm motor neurons, and eventually result in fatality.[111] Variants of SOD1

have been shown to impact copper homeostasis in various organisms. Animal experiments demonstrated that the copper chelator tetrathiomolybdate ammonium effectively decreased the presence of SOD1 aggregates, mitigated the decline of motor neurons and axons, reduced skeletal muscle atrophy in mice, and extended their survival time.[112] Additionally, a study observed diminished copper levels in the cerebrospinal fluid of ALS patients compared with control subjects.[113] Furthermore, when SOD1 is not bound to zinc ions, copper ions in SOD1 facilitate the generation of superoxide anions and subsequent production of peroxynitrite through reaction with nitric oxide.[114] Recent research has provided evidence of a correlation between cuproptosis and the onset of ALS. Swindell et al.[115] conducted

a study that identified differentially expressed genes in ALS through peripheral blood analysis, highlighting the strong association between the gene encoding the superoxide dismutase copper chaperonin and survival rates. Similarly, Jia et al.[116] identified five key predictor genes of ALS (BRCA1 associated deubiquitinase 1, SMG1 nonsense mediated mRNA decay associated PI3K related kinase, BCL2 associated transcription factor 1, DEAH-box helicase 15, eukaryotic translation initiation factor 4 gamma 2) through the analysis of peripheral blood gene expression data using a machine learning model, and successfully developed an support vector machine prediction model with promising results in evaluation. Solovyev et al.[117] identified copper reduction as a distinctive characteristic of ALS through the examination of cerebrospinal fluid. Animal studies have demonstrated that the copper chelator trientine enhances survival in ALS mice.[118] Furthermore, the combination of high doses of trientine and ascorbic acid administered prior to disease onset significantly extended the survival of ALS mice and prevented the deterioration of motor function.[119] Edaravone is currently a primary medication used in the clinical management of ALS, with LeBlanc et al.[120] developing 17 Edaravone analogues that exhibit potent copper-chelating abilities.

4.3 | Huntington disease

HD is a progressive neurodegenerative disorder characterized by dominant polyglutamine amplification at the N-terminus of the huntingtin (HTT) protein.[121] Potential interventions for HD encompass various therapeutic approaches targeting Huntington's DNA and RNA, protein clearance, DNA repair pathways, inflammation, and cellular replacement. However, currently available therapies lack specificity for HD.[122]

Drosophila experiments have demonstrated a correlation between the expression levels of copper metabolism-related genes and the progression of HD. The decrease in copper levels resulted in a significant reduction in the presence of oligomeric and aggregated HTT.[121] Copper has been shown to enhance the formation of thioflavin S-positive Aβ structures within HTT aggregates and impact autophagy levels in the brain. The use of the D-penicillamine (DPA), demonstrated a significant decrease in Aβ aggregation in a Drosophila HD model.[123] Prolonged exposure to copper, zinc, and their combinations has been found to impair locomotor function and cause developmental delays, leading to the aggregation of polyglutamine in both muscle and nerve cells, ultimately resulting in neurodegeneration. On the contrary, rutin demonstrates chelating capabilities in the HD model and serves a protective function in neurodegenerative lesions.[124] Pfalzer

et al.[125] noted an increase in copper levels in cerebrospinal fluid samples at the onset of HD. A comprehensive examination indicates a decrease in copper levels in HD brain tissue.[126] Administration of copper orally was observed to enhance the antioxidant defense mechanism in the rat model with HD, potentially leading to a deceleration of the progression of the disease.[127] Moreover, studies have shown a disruption in copper ion homeostasis in AD, specifically in relation to the proteins Aβ and tau. The gene SOD1 has been identified as playing a crucial role in ALS, with variations in this gene impacting motor neuron function through modulation of copper homeostasis. Furthermore, researchers have identified other genes such as superoxide dismutase copper chaperonin as differentially expressed in these diseases and have developed predictive models for disease progression. Genes involved in copper metabolism have been found to impact levels of HTT, with copper chelators such as DPA and rutin showing potential for improving symptoms of HD.

5 | COPPER HOMEOSTASIS AND CUPROPTOSIS IN LIVER DISEASE

The liver serves as the principal repository for copper in the human body and is integral to copper metabolism. Imbalances in copper levels can lead to oxidative stress, disruption of proteasome function, and impaired angiogenesis, ultimately resulting in cellular damage and the progression of liver disease.[4]

5.1 | Hepatocellular carcinoma

HCC ranks as the fifth most prevalent cancer worldwide and stands as the second leading cause of cancer-related mortality.[128] In recent years, there has been a notable increase in the global incidence of HCC, presenting significant burdens and complexities to the field of global public health.[129] Major risk factors for HCC encompass hepatitis B, hepatitis C, aflatoxin exposure, and alcohol consumption.[130] Additionally, emerging research suggests potential links between metabolic syndrome, nonalcoholic steatohepatitis (NASH), and the development of HCC.[131]

There is already evidence of metabolic disturbances in copper in HCC patients. In a study with 175 HCC patients, serum copper levels exhibited a positive correlation with alanine aminotransferase and the Barcelona stage of HCC.[132] Methylation patterns of CRGs are observed to be differentially upregulated or downregulated in HCC, potentially linked to CRG activation.[133] Gene set variation analysis suggests that CRGs might modulate cuproptosis via metabolic pathways, thereby impacting

HCC development.[134] By prompting DLAT expression and activating the PI3K/mTOR, maternal embryonic leucine zipper kinase enhances mitochondrial function and facilitates HCC.[135] Proteolipid acylation and FDX1 and are essential regulatory genes involved in cuproptosis. Bioinformatics analysis reveals that FDX1 levels are significantly reduced in HCC compared with normal tissues. A higher cuproptosis-related risk score, derived from FDX1, is correlated with shorter patient survival.[136]

Yan et al.[137] developed a prognostic risk model utilizing CRGs (glycine cleavage system protein H, lipoyltransferase 1, and cyclin dependent kinase inhibitor 2A), revealing that downregulation of lipoyltransferase 1 can impede the proliferation and invasion of HCC cells. A prognostic signature associated with cuproptosis (Cu-PS), comprising MAGE family member A6, cytochrome P450, family 26, subfamily b, polypeptide 1, complement C7, erythropoietin, and hexokinase 2, has been identified and analyzed, revealing a significant positive correlation with tumor node metastasis classification, drug sensitivity, tumor mutation load, and response to immunotherapy in HCC. Patients with elevated Cu-PS exhibit a poorer prognosis, particularly those in advanced TNM stages compared with early stages.[138] Chen et al.[139] established a cuproptosis-related signature involving five CRGs-solute carrier family 25 member 28, pyridoxal kinase (PDXK), C-type lectin domain family 3 member B, hepsin, and ring finger protein, transmembrane 1 and observed significantly upregulated expression of PDXK in HCC tissues. Deficiency of PDXK inhibits the proliferation, migration, and invasion of HCC cells.[139] CRGs can modulate HCC development by influencing the tumor immune microenvironment and immune checkpoints. Key genes prion protein, synuclein alpha, and COX17 impact multiple immune cell infiltrations and are notably associated with cytotoxic T lymphocyte antigen 4 and PD-1 levels. Higher expression of prion protein and synuclein alpha fosters immune cell infiltration in HCC with a poorer prognosis, while COX17 suppresses HCC immune cell infiltration.[140] Additionally, cuproptosis-associated lncRNA is closely linked to HCC and serves as a prognostic indicator. The researchers developed a risk assessment model based on HLA complex group 15, AC105020.5, AC079209.1, and AC019069.1, which can be utilized to evaluate the prognosis of HCC.[141]

Sorafenib is the standard treatment for advanced HCC,[142] and patients responsive to treatment exhibit a decrease in cuproptosis-related risk scores.[136] Elesclomol (ES), a copper ion carrier, serves as an inducer of cuproptosis. Studies have encapsulated copper and ES within ROS-sensitive polymers to formulate nanomedicines. Following the translocation into cancer cells, it is released upon ROS induction. ES subsequently triggers cuproptosis and activates the immune response.[143] Angiogenesis

represents a critical hallmark of tumorigenesis, including HCC.[144,145] The copper chelator trientine has been found to inhibit angiogenesis and induce apoptosis of tumor cells, thereby impeding tumor progression.[146] Curcumin potentially inhibits HCC development by reducing the cuproptosis potential index.[147] Furthermore, a risk-prognostic model of CRGs in HCC patients, composed of caudal, sarcoglycan beta, thioredoxin reductase 1, kinase insert domain receptor, and MT-ND4 pseudogene 20, has been established. Patients with high and low scores on this model demonstrate varying sensitivities to different therapeutic drugs for HCC. This observation implies a potential correlation between the expression of CRGs and pharmacological sensitivities in HCC, thereby presenting novel therapeutic strategies for the disease.[148] Emerging evidence indicates that cuproptosis is associated with the progression and prognosis of HCC; however, the precise mechanisms underlying the involvement of cuproptosis in HCC pathogenesis necessitate further investigation (Figure 3).

5.2 | Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological syndrome characterized by the excessive accumulation of fat in hepatocytes, excluding cases attributable to alcohol consumption and other well-defined hepatotoxic factors. It is strongly associated with obesity, insulin resistance, type 2 diabetes mellitus, and dyslipidemia.[149–151] NAFLD has the potential to progress to NASH, which can ultimately lead to cirrhosis and HCC. The global prevalence of NAFLD is approximately 25%, with rates exceeding 30% in South America.[149] NAFLD frequently coexists with other metabolic disorders, presenting a significant public health challenge as its prevalence rises.[152,153]

Porcu et al.[154] observed significantly elevated serum copper levels in patients with NAFLD-cirrhosis, with further increases as the disease advanced to HCC. Mechanistically, heightened serum copper levels regulate the MYC/CTR1 axis, thereby promoting tumor growth, migration, and invasion. CRGs such as NFE2 like bZIP transcription factor 2, DLD, DNA polymerase delta 1, and pyruvate dehydrogenase E1 subunit beta has been found to be potential biomarkers for NAFLD diagnosis.[155] DNA polymerase delta 1 is upregulated in NAFLD and correlates with the cytokine-cytokine receptor interaction pathway. NFE2 like bZIP transcription factor 2 mitigates NAFLD by reducing ROS production, while DLD, highly expressed in NAFLD, enhances ROS generation, exacerbating oxidative stress and NAFLD progression.[156] Additionally, studies have demonstrated high expression of FDX1 in NASH, playing a pivotal role in NASH-HCC development and
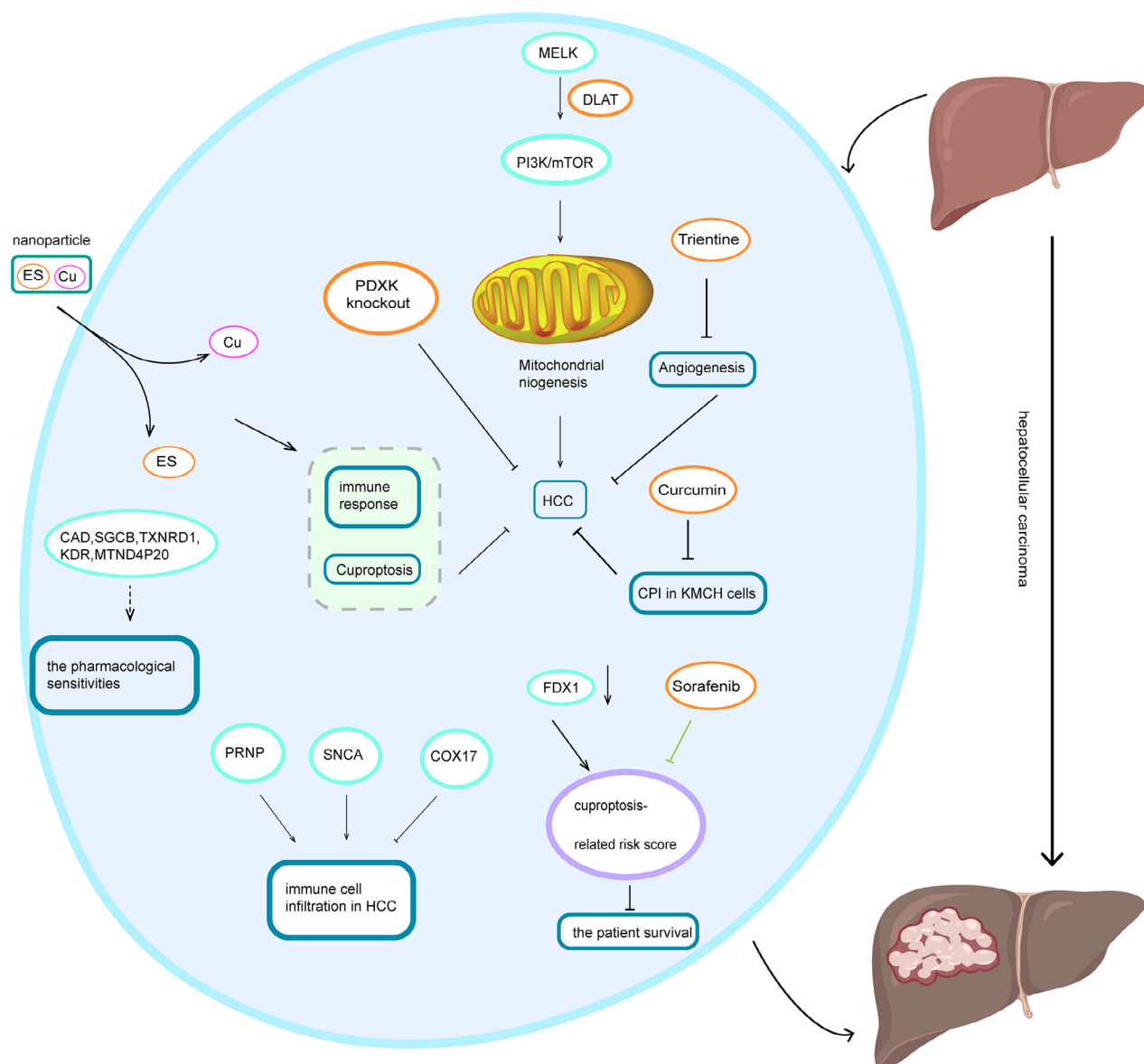
FIGURE 3 Copper homeostasis and cuproptosis in hepatocellular carcinoma. Copper-related genes (CRGs) influence immune cell infiltration. Maternal embryonic leucine zipper kinase (MELK) stabilizes mitochondrial function by activating PI3K/mTOR signaling. Decreased ferredoxin 1 (FDX1) level raises cuproptosis-related risk score, and sorafenib suppresses the score. Curcumin downregulates cuproptosis potential index. Knockdown of pyridoxal kinase (PDXK) inhibits proliferation, migration, and invasion of hepatocellular carcinoma (HCC) cells. Studies have encapsulated Cu and ES with ROS-sensitive polymers, which induces cuproptosis and activates immune response.

providing a new promising therapeutic target for the disease.[157] Copper overload promotes oxidative stress and cellular autophagy, with oxidative stress activating the NFE2 like bZIP transcription factor 2/peroxisome proliferator activated receptor gamma (NRF2/PPARγ) pathway and autophagy mitigating copper-induced lipid deposition, thus offering protection against copper-induced NAFLD.[158,159] However, activation of the NRF2/PPARγ pathway can induce transcription of targeted genes and promote adipogenesis.[160,161] Altered copper levels are associated with the course of NAFLD, but there is still a lack of research on how much of a regulatory role cuproptosis plays in NAFLD and its specific mechanisms (Figure 4).

6 | COPPER HOMEOSTASIS AND CUPROPTOSIS IN HEREDITARY DISEASES

Recent research has elucidated the link between cuproptosis and inherited disorders such as Menkes disease (MD), attributed to copper deficiency,[162] and Wilson's disease
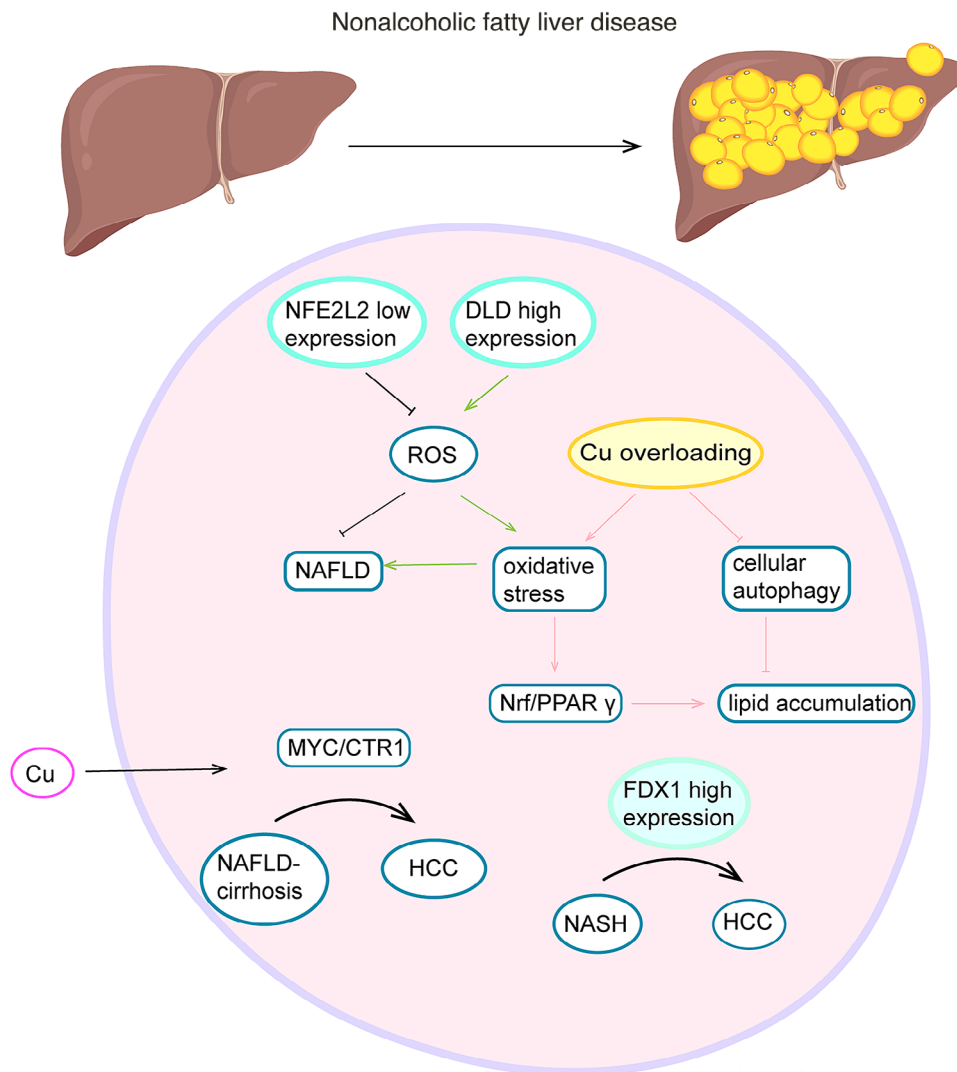
Nonalcoholic fatty liver disease

FIGURE 4 Copper homeostasis and cuproptosis in NAFLD. NFE2 like bZIP transcription factor 2 (NFE2L2) and dihydrolipoamide dehydrogenase (DLD) modulate disease progression through the regulation of reactive oxygen species (ROS) levels. Copper overload influences lipid accumulation, while elevated serum copper levels and ferredoxin 1 (FDX1) promote the transition from nonalcoholic fatty liver disease to hepatocellular carcinoma.

(WD),[163,164] characterized by abnormal copper accumulation. Both conditions are classified as copper storage disorders, emphasizing the importance of restoring copper balance in treatment strategies (Figure 5).

6.1 | Menkes disease

MD is characterized by copper deficiency due to abnormal copper metabolism.[165] The prevalence of MD among live-born boys has been estimated to be one in 35,000.[166] MD is caused by mutations in the ATP7A gene, which disrupts copper homeostasis in nerve tissue, liver, and blood, leading to impaired function of copper-dependent enzymes such as COX, dopamine β-hydroxylase, lysine oxidase, and tyrosinase.[167,168] These disruptions in enzyme func-

tion contribute to the clinical manifestations of the disease, such as stunted growth, psychomotor developmental disorders, spasticity seizures, and various hair abnormalities. A recent study has demonstrated the potential therapeutic effects of the copper-delivering small molecule compound ES in the treatment of MD.[169] Animal studies have shown that ES can improve pathological changes and reduce lethality in Menkes model mice.[170] Furthermore, these studies have indicated that ES facilitates copper transport to mitochondria, leading to increased expression of COX in the brain and mitigation of neurodegenerative lesions.[171] The reduction of ES-Cu(II) to Cu(I) by mitochondrial matrix reductase FDX1 catalyzes the release of copper into the mitochondria, promoting metallation of COX.[172] Animal experiments demonstrated that in lactating and juvenile copper-injected chimeric
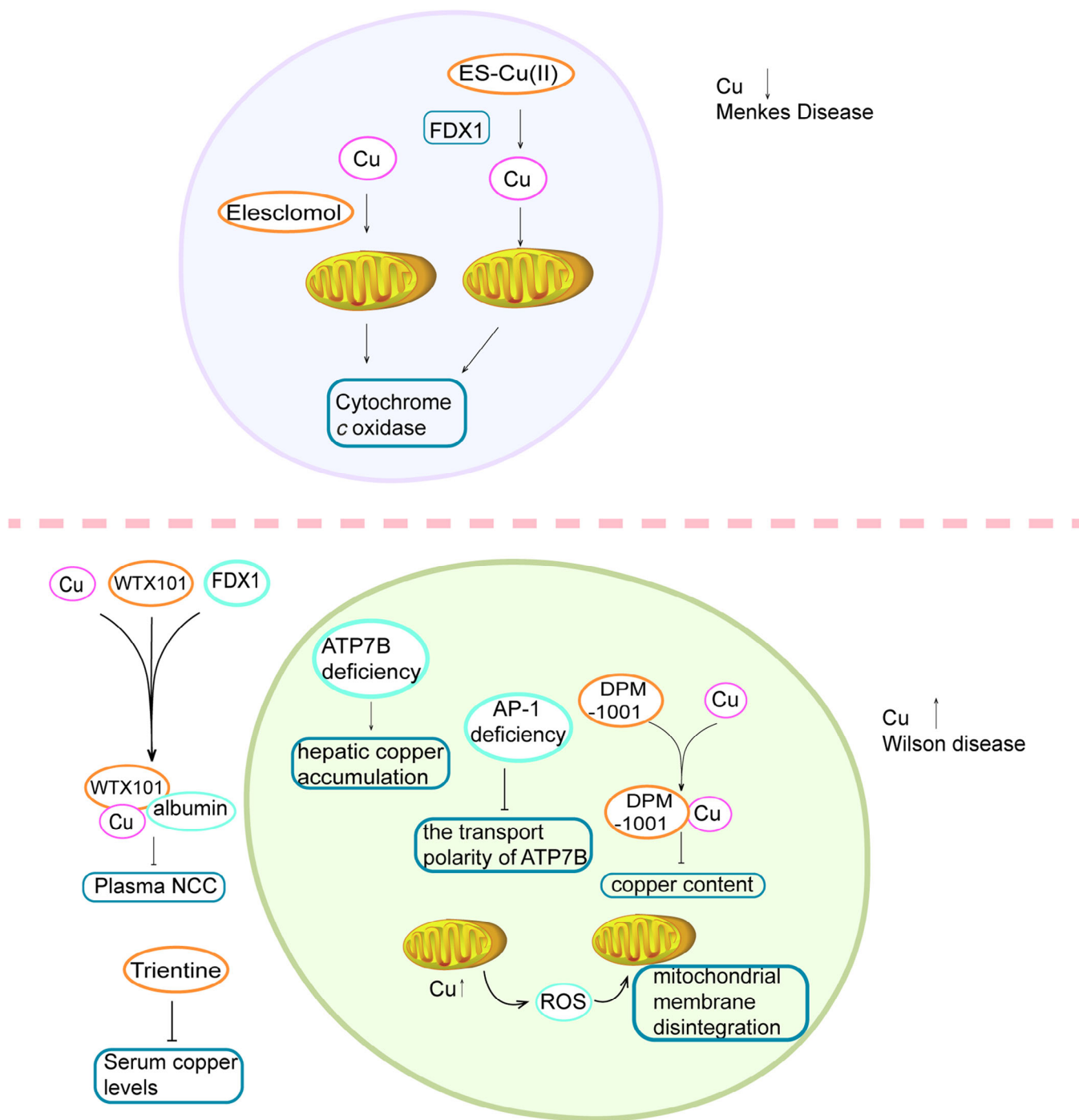
FIGURE 5 Copper homeostasis and cuproptosis in hereditary diseases. In Wilson disease, the deficiency of the adaptor protein (AP-1) hinders the activity of ATPase beta peptide (ATP7B), leading to the accumulation of copper in the liver. Both DMP-1001 and trientine are effective in reducing copper levels. In Menkes disease, the transport of copper to mitochondria, facilitated by the small molecule elesclomol (ES), enhances the expression of cytochrome *c* oxidase in the brain. The reduction of ES-Cu(II) to Cu(I) by ferredoxin 1 (FDX1) catalyzes the metallation of the copper enzyme cytochrome *c* oxidase (COX) within the mitochondria.

mutants, the CTR1 protein translocated from the apical membrane to the cytoplasm of proximal renal tubular epithelial cells, thereby inhibiting copper transport from the primary urine and providing protection against copper toxicity.[173] Additionally, pleckstrin homology domain con-

taining A5 (PLEKHA5), PLEKHA6, and PLEKHA7 recruit PDZ domain containing 11 to distinct plasma membrane locations, facilitating ATP7A targeting to the cell periphery in high copper environments.[174] Furthermore, the use of crystalline nanostructured metal–organic framework

material allows for the controlled release of copper, leading to a reduction in neuronal degeneration and disease progression.[175]

6.2 | Wilson's disease

WD is a rare condition, with an estimated carrier frequency ranging from one in 90 to 150 individuals and an incidence of approximately one in 30,000.[176] Patients with a diagnosis of WD may present with a range of liver-related conditions, including acute liver failure, chronic hepatitis, cirrhosis, and Coombs-negative hemolytic anemia.[176] Liver damage is typically the primary and prevalent manifestation of WD.[163,177] The accumulation of copper in the liver in WD leads to the release of nonfibrin-bound copper into the bloodstream, leading to systemic copper accumulation in organs such as the brain, eyes, and kidneys. Ultimately, this can result in cirrhosis, damage to the basal ganglia, keratoconus, and kidney injury.[178] WD is a hereditary disorder of copper metabolism caused by mutations in the ATP7B gene.[179,180] These mutations result in impaired copper metabolism, as ATP7B is highly expressed in liver tissues. Mutations in ATP7B underlie the pathogenesis of WD, disrupting copper homeostasis.[181,182] Hepatocyte-specific ATP7B deficiency promotes hepatic steatosis and obesity, with hepatic copper accumulation and increased urinary copper excretion.[183] ATP7B, a copper-detoxifying ATPase, demonstrates apical or luminal trafficking polarity. Deficiency of the adaptor protein alters the trafficking polarity of ATP7B in response to intracellular copper levels, suggesting potential implications for the diagnosis and treatment of WD.[184] Proper regulation of copper levels is essential for cellular mitochondrial function, with normal mitochondrial respiration relying on sufficient copper. Excessive hepatic copper levels can negatively impact mitochondrial function, as mitochondria are especially vulnerable to copper overload.[25] Alterations in mitochondrial structure are among the early manifestations of WD.[28] Excessive copper accumulation within mitochondria leads to ROS generation, culminating in mitochondrial membrane disruption and hepatocyte death.[185]

Copper chelators, as cuproptosis reducers, are currently used in the clinical treatment of WD. The main copper chelators that have been entered into clinical studies or approved for treatment of WD include DPA, trientine, bis-choline tetrathiomolybdate. DPA is an United States Food and Drug Administration (US FDA)-approved first-line treatment for WD in the United States; however, approximately one-third of patients experience intolerance to this drug. In 2022, the US FDA approved trientine as an alternative treatment for WD in DPA-intolerant individuals of all ages. A recent study found that trientine was equally as effective as DPA in reducing serum copper levels, with no reported adverse events. Copper chelators, such as trientine, play a crucial role in the clinical management of WD by reducing cuproptosis.[186] Bis-choline tetrathiomolybdate targets intracellular hepatocytic copper and reduces nonceruloplasmin-bound copper by forming a complex with albumin, facilitating copper excretion via bile.[187] A phase 2 clinical trial involving 28 WD patients showed a success rate of 71% with Bis-choline tetrathiomolybdate treatment over 24 weeks; however, 40% of patients experienced liver enzyme elevations.[187] Despite their efficacy, these copper chelators are associated with toxic side effects and necessitate lifelong administration, posing challenges to patient compliance. Consequently, efforts are underway to develop novel copper chelators to broaden WD treatment options and reduce serum copper levels. Chel2 promotes fecal copper excretion in WD mouse models.[188] DPM-1001 exhibits high copper-binding specificity, enhancing cell survival and reducing copper levels, thereby ameliorating WD symptoms.[189] Additionally, studies have demonstrated that methanobactin reverses hepatic injury during acute copper accumulation in the livers of ATP7B-deficient rat.[190] Methanobactin SB2 facilitates excess hepatic copper excretion via the biliary pathway.[191] Current research has clarified the pathogenesis of WD, which is caused by excessive accumulation of copper in the body, and the development of drugs targeting cuproptosis has made some progress and has been applied to clinical treatments.

7 | COPPER HOMEOSTASIS AND CUPROPTOSIS IN CARDIOVASCULAR DISEASE

Research indicates that disruptions in copper homeostasis, specifically in the regulation of copper chaperones or transporters, as well as copper deficiency, play a significant role in the development of various heart diseases.[192] Copper influences heart disease through its impact on mitochondrial function, while dysregulation of copper homeostasis affects FDX1 and lipoic acid synthetase, leading to copper-induced mortality.[193] Cuproptosis is implicated in the pathogenesis of cardiovascular diseases such as atherosclerosis, heart failure, and dilated cardiomyopathy by disrupting lipid balance and promoting oxidative stress, mitochondrial impairment, and dysfunction of endothelial cells[194] (Figure 6).

In the context of atherosclerosis, the upregulation of FDX1 and SLC31A1, along with the downregulation of glutaminase, was observed.[49] FDX1 directly binds to copper, thereby inducing proteotoxic stress, while SLC31A1
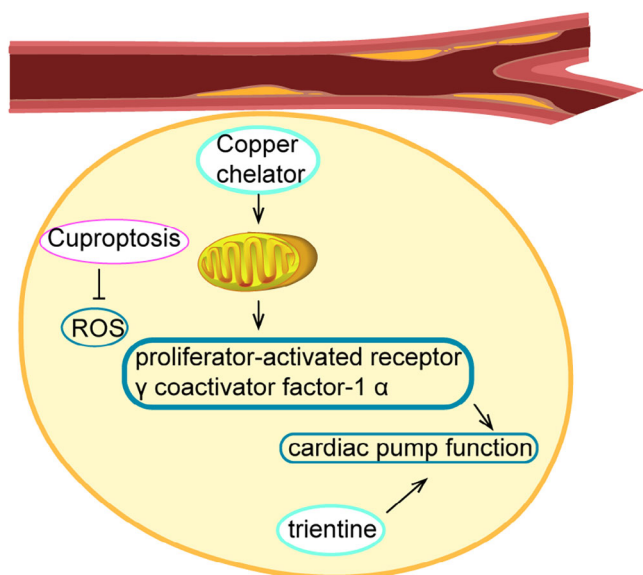
FIGURE 6 Copper homeostasis and cuproptosis in cardiovascular disease. Cuproptosis enhances the accumulation of reactive oxygen species (ROS) in the myocardium. Treatment with a copper chelator has been shown to repair rat myocardial mitochondria and enhance the function of proliferator-activated receptor γ coactivator factor-1 α, ultimately restoring cardiac pumping function. Additionally, trientine has been found to improve cardiac pump function.

facilitates the uptake of copper from the circulation into cells. Glutaminase, on the other hand, enhances intracellular glutathione synthesis to counteract oxidative stress.[195] Wang et al.[196] identified five CRGs and developed a diagnostic model with high diagnostic accuracy based on these findings. Furthermore, two distinct molecular subgroups were identified in atherosclerosis, exhibiting significant differences in mitochondrial outer mem-

brane permeability and primary immunodeficiency.[196] Chen et al.[197] discovered upregulation of SLC31A1 and SLC31A2 expression, as well as downregulation of SOD1 in atherosclerotic plaques, suggesting their potential utility as diagnostic biomarkers for atherosclerosis. Cuproptosis, a process that promotes the accumulation of ROS, is closely linked to the aging process. Elevated levels of copper ions in aging populations have been shown to contribute to vascular senescence and cardiovascular disease.[198] Researchers have developed chiral/phase dual-engineered nanoenzymes that enrich copper and iron atoms in a low-oxidation state when exposed to aging conditions, leading to the onset of cuproptosis. The D-chiral platinum–palladium–copper–iron nanoenzymes have been found to exhibit antiaging and antiatherosclerotic effects in various aging models.[199] Chen et al.[200] formulated a risk assessment model incorporating ten CRGs in congestive heart failure, revealing a significant association between regulatory T cells and the expression levels of pivotal cuproptosis genes such as FDX1, DLD, DLAT, pyruvate dehydrogenase E1 subunit beta, and PDHA1. Subsequently, through rigorous clustering analysis, four key genes (hydroxysteroid dehydrogenase like 2, beta-carotene oxygenase 2, corin, serine peptidase, and small nucleolar RNA, H/ACA box 80A) were identified in diabetes-related heart failure, demonstrating their role in modulating cuproptosis in cardiomyocytes of patients by influencing immune system functionality.[201] Elevated levels of advanced glycosylation end products in diabetic cardiomyopathy contribute to intracellular copper accumulation and the upregulation of the activating transcription factor 3/spi-1 proto-oncogene/SLC31A1 axis, thereby promoting cuproptosis.[202] A study examining the relationship between immune cells and cardiomyopathy-related

TABLE 1 Related drugs targeting copper homeostasis or cuproptosis.

Name	Classification	Diseases	Research and development phase	References
D-Penicillamine	Copper chelator	WD	US/China approved	[186]
Bis-choline tetrathiomolybdate	Copper chelator	WD	Phase 3 clinical trial	[187]
Chel2	Copper chelator	WD	Preclinical studies	[188]
DPM-1001	Copper chelator	WD	Preclinical studies	[189]
Methanobactin	Copper chelator	WD	Preclinical studies	[190]
Sorafenib	Ferroptosis inducer	Advanced HCC	US/EU approved	[142]
Elesclomol	Copper ion carriers	Solid tumor, MD	Phase 1 clinical trial	[143]
Trientine	Copper chelator	HCC, ALS	Preclinical studies	[146]
Clioquinol	Copper chelator	AD	Preclinical studies	[108]
Rutin	Copper chelator	HD	Preclinical studies	[124]
JYFY-001	Copper chelator	CRC	Preclinical studies	[62]
Zinc pyrithione	Copper chelator	Breast cancer	Preclinical studies	[74]

genes in dilated cardiomyopathy revealed a significant positive association between eosinophils and SLC31A1, while DLD exhibited a negative correlation with monocytes.[203] Additionally, six signature genes (septin 1, C-type lectin domain containing 11A, ISG15 ubiquitin like modifier, prolyl 3-hydroxylase 3, serine dehydratase like, and inka box actin regulator 1) linked to immune infiltration were identified as potential diagnostic biomarkers for dilated cardiomyopathy.[204] Several studies have demonstrated the potential of copper chelators to repair rat myocardial mitochondria, enhance myocardial function, and modulate the activity of proliferator-activated receptor γ coactivator factor-1 α, thereby restoring cardiac pumping efficiency.[205] Animal research has also indicated that trientine can enhance cardiac pump function and normalize levels of myocardial copper, copper chaperones, and synthesis of COX1.[206]

8 | CONCLUSION AND PROSPECTS

As one of the trace elements in the human body, copper is essential for the production of various important enzymes, which play pivotal roles in energy production, iron metabolism, antioxidant defense, and the maintenance of normal nerve function.[29] Copper is absorbed through the small intestine and subsequently stored in the liver. Its levels are intricately regulated by a complex network of proteins to maintain a narrow range. Cuproptosis is a novel cell death due to copper overaccumulation.

Perturbations in copper levels have been strongly linked to tumorigenesis, with numerous scholars validating the association between cuproptosis-related genes and various types of cancer, including colorectal, lung, breast, and pancreatic cancers, and developing predictive models based on these findings. Various antitumor drugs aimed at targeting copper homeostasis are currently under development.

Copper mediates the progression of neurodegenerative diseases, and there have been studies confirming the alteration of copper levels in AD, ALS and HD. The key genes have been identified to predict disease progression, and relevant drugs are currently in the process of development.

Given the liver's role as the primary storage organ for copper, the relationship between copper homeostasis and the incidence and progression of liver diseases such as HCC and NAFLD is significant. The levels of CRGs are intricately connected to the pathogenesis of HCC and NAFLD. The use of a risk assessment model based on CRGs can aid in evaluating disease progression, prognosis, and drug responsiveness. Various drugs targeting cuproptosis have been investigated for the treatment of HCC, including sorafenib, ES, and trientine. However, the precise mechanism by which cuproptosis contributes to HCC development requires further elucidation for validation. Furthermore, the existing body of research on copper and NAFLD has predominantly examined the implications of copper deficiency, with a limited number of studies exploring the concept of cuproptosis, which involves excessive copper accumulation, in the development of NAFLD. The majority of current investigations have concentrated on the impact of intracellular copper levels on liver function.

The most pertinent hereditary disorders associated with copper homeostasis are MD and WD, resulting from copper deficiency and excess, respectively. MD is caused by mutations in the ATP7A gene, leading to progressive neurological deterioration and eventual fatality. Treatment options for MD include copper supplementation and supportive care, while advances in gene technology have deepened the study of MD pathogenesis, and the development of CRGs provides new targets for disease treatment. Copper supplementation can elevate copper levels in the body, while copper salt formulations like ES and MOF-74 (Cu) are being developed. WD is a result of impaired copper metabolism due to ATP7B mutations, leading to mitochondrial ultrastructure disruption from copper overload. Current clinical management of WD relies on copper chelators therapy with agents like DPA, bis-choline tetrathiomolybdate, and trientine, which are linked to adverse effects. Novel copper chelators under development, including Chel2, DPM-1001, and methanobactin SB2, seek to mitigate these concerns.

Additionally, copper imbalance and cuproptosis play a role in the pathogenesis of cardiovascular disease, with research indicating a significant association between copper levels and cardiovascular morbidity and mortality. Therefore, restoring copper homeostasis is considered a crucial strategy in the treatment of cardiovascular disease.

Currently, many drugs for metabolic diseases have been developed targeting copper homeostasis and cuproptosis (Table 1).

Research on cuproptosis and its connection to copper homeostasis and disease has advanced significantly and holds promise for further investigation. However, studies investigating the relationship between cuproptosis and various diseases, such as hereditary diseases, liver diseases, neurodegenerative diseases, cancers, and cardiovascular diseases, are still in their nascent stages. The majority of these studies primarily involve correlational analyses between cuproptosis-related genes and disease traits. Nonetheless, there is a notable absence of conclusive evidence to elucidate the regulatory mechanisms of cuproptosis. The complex nature of cuproptosis mechanisms, combined with the lack of definitive biomarkers for monitoring its activity, poses obstacles in the development of inhibitors or inducers for the treatment of liver disease. Additionally, the impact of these interventions on

copper levels in the human body is not well understood, leading to apprehensions regarding potential toxicities. Copper homeostasis and cuproptosis are critical factors in the pathogenesis of numerous diseases, prompting further investigation into the therapeutic potential of targeting copper.

In conclusion, while cuproptosis represents a promising area of research as a recently identified mode of cell death in both physiological and pathological contexts, elucidating its precise mechanisms requires additional study and comprehension.

AUTHOR CONTRIBUTIONS

Design, and supervision; revising of original draft: Yanqi Dang. *Design, and supervision; manuscript editing and revision*: Guang Ji. *Writing of original draft*: Yunuo Yang. *Manuscript editing and revision*: Jiaxuan Wu and Lisheng Wang. The final manuscript has been reviewed and approved by all the authors.

CONFLICT OF INTEREST STATEMENT

All the authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

ETHICS STATEMENT

Not applicable.

ORCID

Yanqi Dang https://orcid.org/0000-0002-0316-7880

REFERENCES

1. Gao L, Zhang A. Copper-instigated modulatory cell mortality mechanisms and progress in oncological treatment investigations. *Front Immunol*. 2023;14:1236063.
2. Blades B, Ayton S, Hung YH, et al. Copper and lipid metabolism: a reciprocal relationship. *Biochim Biophys Acta Gen Subj*. 2021;1865(11):129979.
3. Guan D, Zhao L, Shi X, et al. Copper in cancer: From pathogenesis to therapy. *Biomed Pharmacother*. 2023;163:114791.
4. Liu T, Liu Y, Zhang F, Gao Y. Copper homeostasis dysregulation promoting cell damage and the association with liver diseases. *Chin Med J (Engl)*. 2023;136(14):1653-1662.
5. Yang S, Li Y, Zhou L, et al. Copper homeostasis and cuproptosis in atherosclerosis: metabolism, mechanisms and potential therapeutic strategies. *Cell Death Discov*. 2024;10(1):25.
6. Kim BE, Nevitt T, Thiele DJ. Mechanisms for copper acquisition, distribution and regulation. *Nat Chem Biol*. 2008;4(3):176-185.
7. Ruiz LM, Libedinsky A, Elorza AA. Role of copper on mitochondrial function and metabolism. *Front Mol Biosci*. 2021;8:711227.
8. Das A, Ash D, Fouda AY, et al. Cysteine oxidation of copper transporter CTR1 drives VEGFR2 signalling and angiogenesis. *Nat Cell Biol*. 2022;24(1):35-50.
9. Doguer C, Ha JH, Collins JF. Intersection of iron and copper metabolism in the mammalian intestine and liver. *Compr Physiol*. 2018;8(4):1433-1461.
10. Aigner E, Weiss G, Datz C. Dysregulation of iron and copper homeostasis in nonalcoholic fatty liver. *World J Hepatol*. 2015;7(2):177-188.
11. Arredondo M, Núñez MT. Iron and copper metabolism. *Mol Aspects Med*. 2005;26(4-5):313-327.
12. Wazir SM, Ghobrial I. Copper deficiency, a new triad: anemia, leucopenia, and myeloneuropathy. *J Community Hosp Intern Med Perspect*. 2017;7(4):265-268.
13. Hegazy AA, Zaher MM, Abd El-Hafez MA, et al. Relation between anemia and blood levels of lead, copper, zinc and iron among children. *BMC Res Notes*. 2010;3:133.
14. Collins JF, Prohaska JR, Knutson MD. Metabolic crossroads of iron and copper. *Nutr Rev*. 2010;68(3):133-147.
15. Arredondo M, Martínez R, Núñez MT, et al. Inhibition of iron and copper uptake by iron, copper and zinc. *Biol Res*. 2006;39(1):95-102.
16. Crowe A, Morgan EH. Iron and copper interact during their uptake and deposition in the brain and other organs of developing rats exposed to dietary excess of the two metals. *J Nutr*. 1996;126(1):183-194.
17. Reeves PG, DeMars LC. Copper deficiency reduces iron absorption and biological half-life in male rats. *J Nutr*. 2004;134(8):1953-1957.
18. Arredondo M, Cambiazo V, Tapia L, et al. Copper overload affects copper and iron metabolism in Hep-G2 cells. *Am J Physiol Gastrointest Liver Physiol*. 2004;287(1):G27-G32.
19. Shawki A, Anthony SR, Nose Y, et al. Intestinal DMT1 is critical for iron absorption in the mouse but is not required for the absorption of copper or manganese. *Am J Physiol Gastrointest Liver Physiol*. 2015;309(8):G635-G647.
20. Chen L, Min J, Wang F. Copper homeostasis and cuproptosis in health and disease. *Signal Transduct Target Ther*. 2022;7(1):378.
21. Linder MC. Copper homeostasis in mammals, with emphasis on secretion and excretion. A review. *Int J Mol Sci*. 2020;21(14):4932.
22. Taylor AA, Tsuji JS, Garry MR, et al. Critical review of exposure and effects: implications for setting regulatory health criteria for ingested copper. *Environ Manage*. 2020;65(1):131-159.
23. Tsvetkov PA-O, Coy SA-O, Petrova BA-O, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science*. 2022;375(6586):1254-1261.
24. Gusti AMT, Qusti SY, Alshammari EM, et al. Antioxidants-related superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST),

and nitric oxide synthase (NOS) gene variants analysis in an obese population: a preliminary case-control study. *Antioxidants (Basel)*. 2021;10(4):595.

25. Zischka H, Einer C. Mitochondrial copper homeostasis and its derailment in Wilson disease. *Int J Biochem Cell Biol*. 2018;102:71-75.

26. Trasnea PI, Utz M, Khalfaoui-Hassani B, et al. Cooperation between two periplasmic copper chaperones is required for full activity of the cbb3 -type cytochrome c oxidase and copper homeostasis in Rhodobacter capsulatus. *Mol Microbiol*. 2016;100(2):345-361.

27. Kawamata H, Manfredi G. Import, maturation, and function of SOD1 and its copper chaperone CCS in the mitochondrial intermembrane space. *Antioxid Redox Signal*. 2010;13(9):1375-1384.

28. Blockhuys S, Celauro E, Hildesjö C, et al. Defining the human copper proteome and analysis of its expression variation in cancers. *Metallomics*. 2017;9(2):112-123.

29. Baker ZN, Cobine PA, Leary SC. The mitochondrion: a central architect of copper homeostasis. *Metallomics*. 2017;9(11):1501-1512.

30. Cox TR, Gartland A, Erler JT. Lysyl oxidase, a targetable secreted molecule involved in cancer metastasis. *Cancer Res*. 2016;76(2):188-192.

31. Chen W, Yang A, Jia J, et al. Lysyl oxidase (LOX) family members: rationale and their potential as therapeutic targets for liver fibrosis. *Hepatology*. 2020;72(2):729-741.

32. Johnston KA, Lopez KM. Lysyl oxidase in cancer inhibition and metastasis. *Cancer Lett*. 2018;417:174-181.

33. Al-U'datt D, Allen BG, Nattel S. Role of the lysyl oxidase enzyme family in cardiac function and disease. *Cardiovasc Res*. 2019;115(13):1820-1837.

34. Timón-Gómez A, Nývltová E, Abriata LA, et al. Mitochondrial cytochrome c oxidase biogenesis: recent developments. *Semin Cell Dev Biol*. 2018;76:163-178.

35. Ramzan R, Napiwotzki J, Weber P, et al. Cholate disrupts regulatory functions of cytochrome c oxidase. *Cells*. 2021;10(7):1579.

36. Garza NM, Griffin AT, Zulkifli M, et al. A genome-wide copper-sensitized screen identifies novel regulators of mitochondrial cytochrome c oxidase activity. *J Biol Chem*. 2021;296:100485.

37. Banks CJ, Andersen JL. Mechanisms of SOD1 regulation by post-translational modifications. *Redox Biol*. 2019;26:101270.

38. Tsang CK, Liu Y, Thomas J, et al. Superoxide dismutase 1 acts as a nuclear transcription factor to regulate oxidative stress resistance. *Nat Commun*. 2014;5:3446.

39. Skórska KB, Płaczkowska S, Prescha A, et al. Serum total SOD activity and SOD1/2 concentrations in predicting all-cause mortality in lung cancer patients. *Pharmaceuticals (Basel)*. 2021;14(11):1067.

40. Zhao G, Sun H, Zhang T, Liu JX. Copper induce zebrafish retinal developmental defects via triggering stresses and apoptosis. *Cell Commun Signal*. 2020;18(1):45.

41. Martínez-Reyes I, Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. *Nat Commun*. 2020;11(1):102.

42. Tsvetkov P, Coy S, Petrova B, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science*. 2022;375(6586):1254-1261.

43. Tian Z, Jiang S, Zhou J, Zhang W. Copper homeostasis and cuproptosis in mitochondria. *Life Sci*. 2023;334:122223.

44. Li SR, Bu LL, Cai L. Cuproptosis: lipoylated TCA cycle proteins-mediated novel cell death pathway. *Signal Transduct Target Ther*. 2022;7(1):158.

45. Qin Y, Liu Y, Xiang X, et al. Cuproptosis correlates with immunosuppressive tumor microenvironment based on pan-cancer multiomics and single-cell sequencing analysis. *Mol Cancer*. 2023;22(1):59.

46. Dekker E, Tanis PJ, Vleugels JLA, et al. Colorectal cancer. *Lancet*. 2019;394(10207):1467-1480.

47. Patel SG, Karlitz JJ, Yen T, et al. The rising tide of early-onset colorectal cancer: a comprehensive review of epidemiology, clinical features, biology, risk factors, prevention, and early detection. *Lancet Gastroenterol Hepatol*. 2022;7(3):262-274.

48. Baszuk P, Marciniak W, Derkacz R, et al. Blood copper levels and the occurrence of colorectal cancer in Poland. *Biomedicines*. 2021;9(11):1628.

49. Popovic D, Vucic D, Dikic I. Ubiquitination in disease pathogenesis and treatment. *Nat Med*. 2014;20(11):1242-1253.

50. Squitti R, Pal A, Dhar A, et al. Serum copper status of patients with colorectal cancer: a systematic review and meta-analysis. *J Trace Elem Med Biol*. 2024;82:127370.

51. Ruiz MC, Perelmulter K, Levín P, et al. Antiproliferative activity of two copper (II) complexes on colorectal cancer cell models: Impact on ROS production, apoptosis induction and NF-κB inhibition. *Eur J Pharm Sci*. 2022;169:106092.

52. Shao Y, Fan X, Yang X, et al. Impact of Cuproptosis-related markers on clinical status, tumor immune microenvironment and immunotherapy in colorectal cancer: a multi-omic analysis. *Comput Struct Biotechnol J*. 2023;21:3383-3403.

53. Xu L, Wu P, Rong A, et al. Systematic pan-cancer analysis identifies cuproptosis-related gene DLAT as an immunological and prognostic biomarker. *Aging (Albany NY)*. 2023;15(10):4269-4287.

54. Li L, Sun F, Kong F, et al. Characterization of a cuproptosis-related signature to evaluate immune features and predict prognosis in colorectal cancer. *Front Oncol*. 2023;13:1083956.

55. Gong X, Wu Q, Tan Z, et al. Identification and validation of cuproptosis and disulfidptosis related genes in colorectal cancer. *Cell Signal*. 2024;119:111185.

56. Zhu Z, Zhao Q, Song W, et al. A novel cuproptosis-related molecular pattern and its tumor microenvironment characterization in colorectal cancer. *Front Immunol*. 2022;13:940774.

57. Wang L, Cao Y, Guo W, Xu J. High expression of cuproptosis-related gene FDX1 in relation to good prognosis and immune cells infiltration in colon adenocarcinoma (COAD). *J Cancer Res Clin Oncol*. 2023;149(1):15-24.

58. Wang C, Guo J, Zhang Y, et al. Cuproptosis-related gene FDX1 suppresses the growth and progression of colorectal cancer by retarding EMT progress. *Biochem Genet*. 2024.

59. Wu Z, Lin C, Zhang F, et al. TIGD1 function as a potential cuproptosis regulator following a novel cuproptosis-related gene risk signature in colorectal cancer. *Cancers (Basel)*. 2023;15(8):2286.

60. Yang W, Wang Y, Huang Y, et al. 4-Octyl itaconate inhibits aerobic glycolysis by targeting GAPDH to promote cuproptosis in colorectal cancer. *Biomed Pharmacother*. 2023;159:114301.

61. Zhu Z, Guo T, Weng J, et al. Cuproptosis-related miRNAs signature and immune infiltration characteristics in colorectal cancer. *Cancer Med*. 2023;12(15):16661-16678.

62. Shi X, Li Y, Jia M, et al. A novel copper chelator for the suppression of colorectal cancer. *Drug Dev Res*. 2023;84(2):312-325.

63. Barzaman K, Karami J, Zarei Z, et al. Breast cancer: biology, biomarkers, and treatments. *Int Immunopharmacol*. 2020;84:106535.

64. Britt KL, Cuzick J, Phillips KA. Key steps for effective breast cancer prevention. *Nat Rev Cancer*. 2020;20(8):417-436.

65. Sha S, Si L, Wu X, et al. Prognostic analysis of cuproptosis-related gene in triple-negative breast cancer. *Front Immunol*. 2022;13:922780.

66. Wu X, Zhang Y, Liang G, Ye H. Cuproptosis-related lncRNAs potentially predict prognosis and therapy sensitivity of breast cancer. *Front Pharmacol*. 2023;14:1199883.

67. Wang R, Xu K, Chen Q, et al. Cuproptosis engages in c-Myc-mediated breast cancer stemness. *J Transl Med*. 2023;21(1):409.

68. Qi Y, Yao Q, Li X, et al. Cuproptosis-related gene SLC31A1: prognosis values and potential biological functions in cancer. *Sci Rep*. 2023;13(1):17790.

69. Ning S, Lyu M, Zhu D, et al. Type-I AIE photosensitizer loaded biomimetic system boosting cuproptosis to inhibit breast cancer metastasis and rechallenge. *ACS Nano*. 2023;17(11):10206-10217.

70. Xiong C, Ling H, Hao Q, Zhou X. Cuproptosis: p53-regulated metabolic cell death? *Cell Death Differ*. 2023;30(4):876-884.

71. Wu JH, Cheng TC, Zhu B, et al. Identification of cuproptosis-related gene SLC31A1 and upstream LncRNA-miRNA regulatory axis in breast cancer. *Sci Rep*. 2023;13(1):18390.

72. Liu Y, Wang J, Jiang M. Copper-related genes predict prognosis and characteristics of breast cancer. *Front Immunol*. 2023;14:1145080.

73. Sha R, Dong X, Yan S, et al. Cuproptosis-related genes predict prognosis and trastuzumab therapeutic response in HER2-positive breast cancer. *Sci Rep*. 2024;14(1):2908.

74. Yang X, Deng L, Diao X, et al. Targeting cuproptosis by zinc pyrithione in triple-negative breast cancer. *iScience*. 2023;26(11):108218.

75. Wang C, Tan S, Li J, et al. CircRNAs in lung cancer - Biogenesis, function and clinical implication. *Cancer Lett*. 2020;492:106-115.

76. The L. Lung cancer: some progress, but still a lot more to do. *Lancet*. 2019;394(10212):1880.

77. Thai AA, Solomon BJ, Sequist LV, et al. Lung cancer. *Lancet*. 2021;398(10299):535-554.

78. Zhang W, Qu H, Ma X, et al. Identification of cuproptosis and immune-related gene prognostic signature in lung adenocarcinoma. *Front Immunol*. 2023;14:1179742.

79. Chen Y, Tang L, Huang W, et al. Identification and validation of a novel cuproptosis-related signature as a prognostic model for lung adenocarcinoma. *Front Endocrinol (Lausanne)*. 2022;13:963220.

80. Xiaona X, Liu Q, Zhou X, et al. Comprehensive analysis of cuproptosis-related genes in immune infiltration and prognosis in lung adenocarcinoma. *Comput Biol Med*. 2023;158:106831.

81. Yang J, Liu K, Yang L, et al. Identification and validation of a novel cuproptosis-related stemness signature to predict prognosis and immune landscape in lung adenocarcinoma by integrating single-cell and bulk RNA-sequencing. *Front Immunol*. 2023;14:1174762.

82. Wang X, Jing H, Li H. A novel cuproptosis-related lncRNA signature to predict prognosis and immune landscape of lung adenocarcinoma. *Transl Lung Cancer Res*. 2023;12(2):230-246.

83. Ma C, Li F, He Z, et al. Prognosis and personalized treatment prediction in lung adenocarcinoma: an in silico and in vitro strategy adopting cuproptosis related lncRNA towards precision oncology. *Front Pharmacol*. 2023;14:1113808.

84. Deng L, Jiang A, Zeng H, et al. Comprehensive analyses of PDHA1 that serves as a predictive biomarker for immunotherapy response in cancer. *Front Pharmacol*. 2022;13:947372.

85. Tang Y, Wang T, Li Q, Shi J. A cuproptosis score model and prognostic score model can evaluate clinical characteristics and immune microenvironment in NSCLC. *Cancer Cell Int*. 2024;24(1):68.

86. Wang W, Lu K, Jiang X, et al. Ferroptosis inducers enhanced cuproptosis induced by copper ionophores in primary liver cancer. *J Exp Clin Cancer Res*. 2023;42(1):142.

87. Li A, Huang K, Pan W, et al. Thiosemicarbazone mixed-valence Cu(I/II) complex against lung adenocarcinoma cells through multiple pathways involving cuproptosis. *J Med Chem*. 2024;67(11):9091-9103.

88. Tang W, Wu J, Wang L, et al. Bioactive layered double hydroxides for synergistic sonodynamic/cuproptosis anticancer therapy with elicitation of the immune response. *ACS Nano*. 2024;18(15):10495-10508.

89. Stoffel EM, Brand RE, Goggins M. Pancreatic cancer: changing epidemiology and new approaches to risk assessment, early detection, and prevention. *Gastroenterology*. 2023;164(5):752-765.

90. Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. *Nat Rev Gastroenterol Hepatol*. 2021;18(7):493-502.

91. Yao HF, Xu DP, Zheng JH, et al. Analysis of cuproptosis-related lncRNA signature for predicting prognosis and tumor immune microenvironment in pancreatic cancer. *Apoptosis*. 2023;28(7-8):1090-1112.

92. Sun Y, Yao L, Man C, et al. Development and validation of cuproptosis-related lncRNAs associated with pancreatic cancer immune microenvironment based on single-cell. *Front Immunol*. 2023;14:1220760.

93. Chen L, Zhang L, He H, et al. Systemic analyses of cuproptosis-related lncRNAs in pancreatic adenocarcinoma, with a focus on the molecular mechanism of LINC00853. *Int J Mol Sci*. 2023;24(9):7923.

94. Zhang X, Zhou Y, Hu J, et al. Comprehensive analysis identifies cuproptosis-related gene DLAT as a potential prognostic and immunological biomarker in pancreatic adenocarcinoma. *BMC Cancer*. 2023;23(1):560.

95. Yu Q, Zhou J, Liu Y, et al. DNAzyme-mediated cascade nanoreactor for cuproptosis-promoted pancreatic cancer synergistic therapy. *Adv Healthc Mater*. 2023;12(28):e2301429.

96. Hu F, Huang J, Bing T, et al. Stimulus-responsive copper complex nanoparticles induce cuproptosis for augmented cancer immunotherapy. *Adv Sci (Weinh)*. 2024;11(13):e2309388.

97. Yu N, Zhou J, Ding M, et al. Sono-triggered cascade lactate depletion by semiconducting polymer nanoreactors for cuproptosis-immunotherapy of pancreatic cancer. *Angew Chem Int Ed Engl*. 2024;63(30):e202405639.

98. Xiao C, Li J, Hua A, et al. Hyperbaric oxygen boosts anti-tumor efficacy of copper-diethyldithiocarbamate nanoparticles against pancreatic ductal adenocarcinoma by regulating cancer stem cell metabolism. *Research (Wash D C)*. 2024;7: 0335.

99. Bandmann O, Weiss KH, Kaler SG. Wilson's disease and other neurological copper disorders. *Lancet Neurol*. 2015;14(1):103-113.

100. Wang Q, Sun J, Chen T, et al. Ferroptosis, pyroptosis, and cuproptosis in Alzheimer's disease. *ACS Chem Neurosci*. 2023;14(19):3564-3587.

101. Scheltens P, De Strooper B, Kivipelto M, et al. Alzheimer's disease. *Lancet*. 2021;397(10284):1577-1590.

102. Ma C, Hong F, Yang S. Amyloidosis in Alzheimer's disease: pathogeny, etiology, and related therapeutic directions. *Molecules*. 2022;27(4):1210.

103. Chen LL, Fan YG, Zhao LX, et al. The metal ion hypothesis of Alzheimer's disease and the anti-neuroinflammatory effect of metal chelators. *Bioorg Chem*. 2023;131:106301.

104. Tiwari S, Atluri V, Kaushik A, et al. Alzheimer's disease: pathogenesis, diagnostics, and therapeutics. *Int J Nanomedicine*. 2019;14:5541-5554.

105. Li K, Li A, Mei Y, et al. Trace elements and Alzheimer dementia in population-based studies: a bibliometric and meta-analysis. *Environ Pollut*. 2023;318:120782.

106. Squitti R, Ventriglia M, Simonelli I, et al. Copper imbalance in Alzheimer's disease: meta-analysis of serum, plasma, and brain specimens, and replication study evaluating ATP7B gene variants. *Biomolecules*. 2021;11(7):960.

107. Singh SK, Balendra V, Obaid AA, et al. Copper-mediated *β*-amyloid toxicity and its chelation therapy in Alzheimer's disease. *Metallomics*. 2022;14(6):mfac018.

108. Regland B, Lehmann W, Abedini I, et al. Treatment of Alzheimer's disease with clioquinol. *Dement Geriatr Cogn Disord*. 2001;12(6):408-414.

109. Al-Chalabi A, Hardiman O. The epidemiology of ALS: a conspiracy of genes, environment and time. *Nat Rev Neurol*. 2013;9(11):617-628.

110. Feldman EL, Goutman SA, Petri S, et al. Amyotrophic lateral sclerosis. *Lancet*. 2022;400(10360):1363-1380.

111. Tafuri F, Ronchi D, Magri F, et al. SOD1 misplacing and mitochondrial dysfunction in amyotrophic lateral sclerosis pathogenesis. *Front Cell Neurosci*. 2015;9:336.

112. Tokuda E, Okawa E, Watanabe S, et al. Dysregulation of intracellular copper homeostasis is common to transgenic mice expressing human mutant superoxide dismutase-1s regardless of their copper-binding abilities. *Neurobiol Dis*. 2013;54:308-319.

113. Chen QY, Wu P, Wen T, et al. Association of cerebral spinal fluid copper imbalance in amyotrophic lateral sclerosis. *Front Aging Neurosci*. 2022;14:970711.

114. Furukawa Y, O'Halloran TV. Posttranslational modifications in Cu,Zn-superoxide dismutase and mutations associated with amyotrophic lateral sclerosis. *Antioxid Redox Signal*. 2006;8(5-6):847-867.

115. Swindell WR, Kruse CPS, List EO, et al. ALS blood expression profiling identifies new biomarkers, patient subgroups, and evidence for neutrophilia and hypoxia. *J Transl Med*. 2019;17(1):170.

116. Jia F, Zhang B, Yu W, et al. Exploring the cuproptosis-related molecular clusters in the peripheral blood of patients with amyotrophic lateral sclerosis. *Comput Biol Med*. 2024;168:107776.

117. Solovyev N, Lucio M, Mandrioli J, et al. Interplay of Metallome and Metabolome in Amyotrophic Lateral Sclerosis: A Study on Cerebrospinal Fluid of Patients Carrying Disease-Related Gene Mutations. *ACS Chem Neurosci*. 2023;14(17):3035-3046.

118. Andreassen OA, Dedeoglu A, Friedlich A, et al. Effects of an inhibitor of poly(ADP-ribose) polymerase, desmethylselegiline, trientine, and lipoic acid in transgenic ALS mice. *Exp Neurol*. 2001;168(2):419-424.

119. Nagano S, Fujii Y, Yamamoto T, et al. The efficacy of trientine or ascorbate alone compared to that of the combined treatment with these two agents in familial amyotrophic lateral sclerosis model mice. *Exp Neurol*. 2003;179(2):176-180.

120. LeBlanc A, Cuperlovic-Culf M, Morin PJ, Touaibia M. Structurally Related Edaravone Analogues: Synthesis, Antiradical, Antioxidant, and Copper-Chelating Properties. *CNS Neurol Disord Drug Targets*. 2019;18(10):779-790.

121. Xiao G, Fan Q, Wang X, Zhou B. Huntington disease arises from a combinatory toxicity of polyglutamine and copper binding. *Proc Natl Acad Sci U S A*. 2013;110(37):14995-5000.

122. Tabrizi SJ, Estevez-Fraga C, van Roon-Mom WMC, et al. Potential disease-modifying therapies for Huntington's disease: lessons learned and future opportunities. *Lancet Neurol*. 2022;21(7):645-658.

123. Lobato AG, Ortiz-Vega N, Zhu Y, et al. Copper enhances aggregational toxicity of mutant huntingtin in a Drosophila model of Huntington's Disease. *Biochim Biophys Acta Mol Basis Dis*. 2024;1870(1):166928.

124. Cordeiro LM, Soares MV, da Silva AF, et al. Toxicity of copper and zinc alone and in combination in Caenorhabditis elegans model of Huntington's disease and protective effects of rutin. *Neurotoxicology*. 2023;97:120-132.

125. Pfalzer AC, Yan Y, Kang H, et al. Alterations in metal homeostasis occur prior to canonical markers in Huntington disease. *Sci Rep*. 2022;12(1):10373.

126. Scholefield M, Unwin RD, Cooper GJS. Shared perturbations in the metallome and metabolome of Alzheimer's, Parkinson's, Huntington's, and dementia with Lewy bodies: a systematic review. *Ageing Res Rev*. 2020;63:101152.

127. Martínez-Lazcano JC, Montes S, Sánchez-Mendoza MA, et al. Sub-chronic copper pretreatment reduces oxidative damage in an experimental Huntington's disease model. *Biol Trace Elem Res*. 2014;162(1-3):211-218.

128. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249.

129. EASL clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol*. 2018;69(1):182-236.

130. Akinyemiju T, Abera S, Ahmed M, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. *JAMA Oncol*. 2017;3(12):1683-1691.

131. Shah PA, Patil R, Harrison SA. NAFLD-related hepatocellular carcinoma: the growing challenge. *Hepatology*. 2023;77(1):323-338.

132. Tamai Y, Iwasa M, Eguchi A, et al. Serum copper, zinc and metallothionein serve as potential biomarkers for hepatocellular carcinoma. *PLoS One*. 2020;15(8):e0237370.

133. Qin H, Sheng W, Zhang G, et al. Comprehensive analysis of cuproptosis-related prognostic gene signature and tumor immune microenvironment in HCC. *Front Genet*. 2023;14:1094793.

134. Wang Y, Zhang Y, Wang L, et al. Development and experimental verification of a prognosis model for cuproptosis-related subtypes in HCC. *Hepatol Int*. 2022;16(6):1435-1447.

135. Li Z, Zhou H, Zhai X, et al. MELK promotes HCC carcinogenesis through modulating cuproptosis-related gene DLAT-mediated mitochondrial function. *Cell Death Dis*. 2023;14(11):733.

136. Zhang Z, Zeng X, Wu Y, et al. Cuproptosis-related risk score predicts prognosis and characterizes the tumor microenvironment in hepatocellular carcinoma. *Front Immunol*. 2022;13:925618.

137. Yan C, Niu Y, Ma L, et al. System analysis based on the cuproptosis-related genes identifies LIPT1 as a novel therapy target for liver hepatocellular carcinoma. *J Transl Med*. 2022;20(1):452.

138. Wang XX, Wu LH, Ji H, et al. A novel cuproptosis-related prognostic signature and potential value in HCC immunotherapy. *Front Mol Biosci*. 2022;9:1001788.

139. Chen Y, Tang L, Huang W, et al. Identification of a prognostic cuproptosis-related signature in hepatocellular carcinoma. *Biol Direct*. 2023;18(1):4.

140. Wang X, Chen D, Shi Y, et al. Copper and cuproptosis-related genes in hepatocellular carcinoma: therapeutic biomarkers targeting tumor immune microenvironment and immune checkpoints. *Front Immunol*. 2023;14:1123231.

141. Chen S, Liu P, Zhao L, et al. A novel cuproptosis-related prognostic lncRNA signature for predicting immune and drug therapy response in hepatocellular carcinoma. *Front Immunol*. 2022;13:954653.

142. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet*. 2018;391(10127):1301-1314.

143. Guo B, Yang F, Zhang L, et al. Cuproptosis induced by ros responsive nanoparticles with elesclomol and copper combined with αPD-L1 for enhanced cancer immunotherapy. *Adv Mater*. 2023;35(22):e2212267.

144. Moawad AW, Szklaruk J, Lall C, et al. Angiogenesis in hepatocellular carcinoma; pathophysiology, targeted therapy, and role of imaging. *J Hepatocell Carcinoma*. 2020;7:77-89.

145. Fodor D, Jung I, Turdean S, et al. Angiogenesis of hepatocellular carcinoma: an immunohistochemistry study. *World J Hepatol*. 2019;11(3):294-304.

146. Yoshii J, Yoshiji H, Kuriyama S, et al. The copper-chelating agent, trientine, suppresses tumor development and angiogenesis in the murine hepatocellular carcinoma cells. *Int J Cancer*. 2001;94(6):768-773.

147. Liu Z, Ma H, Lai Z. The role of ferroptosis and cuproptosis in curcumin against hepatocellular carcinoma. *Molecules*. 2023;28(4):1623.

148. Xu S, Dong K, Gao R, et al. Cuproptosis-related signature for clinical prognosis and immunotherapy sensitivity in hepatocellular carcinoma. *J Cancer Res Clin Oncol*. 2023;149(13):12249-12263.

149. Paschos P, Paletas K. Non alcoholic fatty liver disease and metabolic syndrome. *Hippokratia*. 2009;13(1):9-19.

150. Guo X, Yin X, Liu Z, Wang J. Non-alcoholic fatty liver disease (NAFLD) pathogenesis and natural products for prevention and treatment. *Int J Mol Sci*. 2022;23(24):15489.

151. Godoy-Matos AF, Silva Junior WS, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. *Diabetol Metab Syndr*. 2020;12:60.

152. Younossi ZM. Non-alcoholic fatty liver disease - a global public health perspective. *J Hepatol*. 2019;70(3):531-544.

153. Cotter TG, Rinella M. Nonalcoholic fatty liver disease 2020: the state of the disease. *Gastroenterology*. 2020;158(7):1851-1864.

154. Porcu C, Antonucci L, Barbaro B, et al. Copper/MYC/CTR1 interplay: a dangerous relationship in hepatocellular carcinoma. *Oncotarget*. 2018;9(10):9325-9343.

155. Wu C, Liu X, Zhong L, et al. Identification of cuproptosis-related genes in nonalcoholic fatty liver disease. *Oxid Med Cell Longev*. 2023;2023:9245667.

156. Ouyang G, Wu Z, Liu Z, et al. Identification and validation of potential diagnostic signature and immune cell infiltration for NAFLD based on cuproptosis-related genes by bioinformatics analysis and machine learning. *Front Immunol*. 2023;14:1251750.

157. Zhao Z, Luan T, Wan J, et al. Elucidating cuproptosis-associated genes in the progression from nash to HCC using bulk and single-cell RNA sequencing analyses and experimental validation. *Medicina (Kaunas)*. 2023;59(9):1639.

158. Zhong CC, Zhao T, Hogstrand C, et al. Copper (Cu) induced changes of lipid metabolism through oxidative stress-mediated autophagy and Nrf2/PPARgamma pathways. *J Nutr Biochem*. 2022;100:108883.

159. Polvani S, Tarocchi M, Galli A. PPARgamma and oxidative stress: con(beta) catenating NRF2 and FOXO. *PPAR Res*. 2012;2012:641087.

160. Zhong CC, Zhao T, Hogstrand C, et al. Copper (Cu) induced changes of lipid metabolism through oxidative stress-mediated autophagy and Nrf2/PPARγ pathways. *J Nutr Biochem*. 2022;100:108883.

161. Mota de Sa P, Richard AJ, Hang H, Stephens JM. Transcriptional regulation of adipogenesis. *Compr Physiol*. 2017;7(2):635-674.

162. Horn N, Møller LB, Nurchi VM, Aaseth J. Chelating principles in Menkes and Wilson diseases: choosing the right compounds in the right combinations at the right time. *J Inorg Biochem*. 2019;190:98-112.

163. Członkowska A, Litwin T, Dusek P, et al. Wilson disease. *Nat Rev Dis Primers*. 2018;4(1):21.

164. Shribman S, Marjot T, Sharif A, et al. Investigation and management of Wilson's disease: a practical guide from the British Association for the Study of the Liver. *Lancet Gastroenterol Hepatol*. 2022;7(6):560-575.

165. Tümer Z, Møller LB. Menkes disease. *Eur J Hum Genet*. 2010;18(5):511-518.

166. Springer C, Humayun D, Skouta R. Cuproptosis: unraveling the mechanisms of copper-induced cell death and its implication in cancer therapy. *Cancers (Basel)*. 2024;16(3):647.

167. Horn N, Wittung-Stafshede P. ATP7A-regulated enzyme metalation and trafficking in the menkes disease puzzle. *Biomedicines*. 2021;9(4):391.

168. Ahuja A, Dev K, Tanwar RS, et al. Copper mediated neurological disorder: visions into amyotrophic lateral sclerosis, Alzheimer and Menkes disease. *J Trace Elem Med Biol.* 2015;29:11-23.

169. Garza NM, Swaminathan AB, Maremanda KP, et al. Mitochondrial copper in human genetic disorders. *Trends Endocrinol Metab.* 2023;34(1):21-33.

170. Yuan S, Korolnek T, Kim BE. Oral elesclomol treatment alleviates copper deficiency in animal models. *Front Cell Dev Biol.* 2022;10:856300.

171. Guthrie LM, Soma S, Yuan S, et al. Elesclomol alleviates Menkes pathology and mortality by escorting Cu to cuproenzymes in mice. *Science.* 2020;368(6491):620-625.

172. Zulkifli M, Spelbring AN, Zhang Y, et al. FDX1-dependent and independent mechanisms of elesclomol-mediated intracellular copper delivery. *Proc Natl Acad Sci U S A.* 2023;120(10):e2216722120.

173. Haberkiewicz O, Lipiński P, Starzyński RR, et al. Decreased expression of the Slc31a1 gene and cytoplasmic relocalization of membrane CTR1 protein in renal epithelial cells: a potent protective mechanism against copper nephrotoxicity in a mouse model of menkes disease. *Int J Mol Sci.* 2022;23(19): 11441.

174. Sluysmans S, Méan I, Xiao T, et al. PLEKHA5, PLEKHA6, and PLEKHA7 bind to PDZD11 to target the Menkes ATPase ATP7A to the cell periphery and regulate copper homeostasis. *Mol Biol Cell.* 2021;32(21):ar34.

175. Aguila-Rosas J, García-Martínez BA, Ríos C, et al. Copper release by MOF-74(Cu): a novel pharmacological alternative to diseases with deficiency of a vital oligoelement. *RSC Adv.* 2024;14(2):855-862.

176. EASL clinical practice guidelines: Wilson's disease. *J Hepatol.* 2012;56(3):671-685.

177. Schilsky ML. Wilson disease: clinical manifestations, diagnosis, and treatment. *Clin Liver Dis (Hoboken).* 2014;3(5):104-107.

178. Shribman S, Poujois A, Bandmann O, et al. Wilson's disease: update on pathogenesis, biomarkers and treatments. *J Neurol Neurosurg Psychiatry.* 2021;92(10):1053-1061.

179. Lorincz MT. Neurologic Wilson's disease. *Ann N Y Acad Sci.* 2010;1184:173-187.

180. Kaler SG. Inborn errors of copper metabolism. *Handb Clin Neurol.* 2013;113:1745-1754.

181. Pierson H, Muchenditsi A, Kim BE, et al. The function of ATPase copper transporter ATP7B in intestine. *Gastroenterology.* 2018;154(1):168-180. e5.

182. Polishchuk EV, Concilli M, Iacobacci S, et al. Wilson disease protein ATP7B utilizes lysosomal exocytosis to maintain copper homeostasis. *Dev Cell.* 2014;29(6):686-700.

183. Muchenditsi A, Yang H, Hamilton JP, et al. Targeted inactivation of copper transporter Atp7b in hepatocytes causes liver steatosis and obesity in mice. *Am J Physiol Gastrointest Liver Physiol.* 2017;313(1):G39-G49.

184. Ruturaj, Mishra M, Saha S, et al. Regulation of the apico-basolateral trafficking polarity of the homologous copper-ATPases ATP7A and ATP7B. *J Cell Sci.* 2024;137(5): jcs261258.

185. Zischka H, Lichtmannegger J. Pathological mitochondrial copper overload in livers of Wilson's disease patients and related animal models. *Ann N Y Acad Sci.* 2014;1315:6-15.

186. Schilsky ML, Czlonkowska A, Zuin M, et al. Trientine tetrahydrochloride versus penicillamine for maintenance therapy in Wilson disease (CHELATE): a randomised, open-label, non-inferiority, phase 3 trial. *Lancet Gastroenterol Hepatol.* 2022;7(12):1092-1102.

187. Weiss KH, Askari FK, Czlonkowska A, et al. Bis-choline tetrathiomolybdate in patients with Wilson's disease: an open-label, multicentre, phase 2 study. *Lancet Gastroenterol Hepatol.* 2017;2(12):869-876.

188. Monestier M, Pujol AM, Lamboux A, et al. A liver-targeting Cu(i) chelator relocates Cu in hepatocytes and promotes Cu excretion in a murine model of Wilson's disease. *Metallomics.* 2020;12(6):1000-1008.

189. Krishnan N, Felice C, Rivera K, et al. DPM-1001 decreased copper levels and ameliorated deficits in a mouse model of Wilson's disease. *Genes Dev.* 2018;32(13-14):944-952.

190. Lichtmannegger J, Leitzinger C, Wimmer R, et al. Methanobactin reverses acute liver failure in a rat model of Wilson disease. *J Clin Invest.* 2016;126(7):2721-2735.

191. Einer C, Munk DE, Park E, et al. ARBM101 (methanobactin SB2) drains excess liver copper via biliary excretion in Wilson's disease rats. *Gastroenterology.* 2023;165(1):187-200. e7.

192. Liu Y, Miao J. An emerging role of defective copper metabolism in heart disease. *Nutrients.* 2022;14(3):700.

193. Parsanathan R. Copper's dual role: unravelling the link between copper homeostasis, cuproptosis, and cardiovascular diseases. *Hypertens Res.* 2024;47(5):1440-1442.

194. Chen X, Cai Q, Liang R, et al. Copper homeostasis and copper-induced cell death in the pathogenesis of cardiovascular disease and therapeutic strategies. *Cell Death Dis.* 2023;14(2):105.

195. Cui Y, Chen Y, Gan N, et al. A novel cuproptosis-related diagnostic gene signature and differential expression validation in atherosclerosis. *Mol Biomed.* 2023;4(1):21.

196. Wang M, Cheng L, Xiang Q, et al. Evaluation the role of cuproptosis-related genes in the pathogenesis, diagnosis and molecular subtypes identification of atherosclerosis. *Heliyon.* 2023;9(10):e21158.

197. Chen YT, Xu XH, Lin L, et al. Identification of three cuproptosis-specific expressed genes as diagnostic biomarkers and therapeutic targets for atherosclerosis. *Int J Med Sci.* 2023;20(7):836-848.

198. Chen Z, Li YY, Liu X. Copper homeostasis and copper-induced cell death: Novel targeting for intervention in the pathogenesis of vascular aging. *Biomed Pharmacother.* 2023;169:115839.

199. Ding C, Min J, Tan Y, et al. Combating atherosclerosis with chirality/phase dual-engineered nanozyme featuring microenvironment-programmed senolytic and senomorphic actions. *Adv Mater.* 2024;36(29):e2401361.

200. Chen Z, Zhu Y, Chen S, et al. Immune patterns of cuproptosis in ischemic heart failure: a transcriptome analysis. *J Cell Mol Med.* 2024;28(7):e18187.

201. Chen J, Yang X, Li W, et al. Potential molecular and cellular mechanisms of the effects of cuproptosis-related genes in the cardiomyocytes of patients with diabetic heart failure: a bioinformatics analysis. *Front Endocrinol (Lausanne).* 2024;15:1370387.

202. Huo S, Wang Q, Shi W, et al. ATF3/SPI1/SLC31A1 signaling promotes cuproptosis induced by advanced glycosylation

end products in diabetic myocardial injury. *Int J Mol Sci.* 2023;24(2):1667.

203. Bian R, Wang Y, Li Z, Xu X. Identification of cuproptosis-related biomarkers in dilated cardiomyopathy and potential therapeutic prediction of herbal medicines. *Front Mol Biosci.* 2023;10:1154920.

204. Lin Y, Chen K, Guo J, et al. Identification of cuproptosis-related genes and immune infiltration in dilated cardiomyopathy. *Int J Cardiol.* 2024;399:131702.

205. Wang D, Tian Z, Zhang P, et al. The molecular mechanisms of cuproptosis and its relevance to cardiovascular disease. *Biomed Pharmacother.* 2023;163:114830.

206. Zhang S, Liu H, Amarsingh GV, et al. Restoration of myocellular copper-trafficking proteins and mitochondrial copper enzymes repairs cardiac function in rats with diabetes-evoked heart failure. *Metallomics.* 2020;12(2):259-272.