Role and mechanisms of cuproptosis in the pathogenesis of Wilson's disease (Review)

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Abstract. Copper, an indispensable trace element in living organisms, plays a pivotal role in human physiological processes. Wilson's disease (WD), an inherited disorder of copper metabolism, is caused by mutations in the ATP7B gene. This genetic malfunction disrupts the dynamics of copper transport and metabolism, thereby impairing ceruloplasmin synthesis and copper excretion. The resultant accumulation of copper in various tissues and organs precipitates a cascade of cellular demise and functional impairment. Notably, cuproptosis, a recently discovered copper-dependent regulated cell death mechanism, distinctly deviates from conventional cell death paradigms. This novel mode of cell death involves the interaction of copper with lipoacylated proteins within the tricarboxylic acid cycle, leading to proteinotoxic stress and culminating in cell death. In the realm of pathophysiology, cuproptosis has emerged as a pivotal player in a spectrum of diseases, with WD standing as a paradigm closely intertwined with the dysregulation of copper metabolism. This study aimed to encapsulate the pivotal molecular underpinnings of cuproptosis and delve into its crucial involvement in the etiopathogenesis of WD. By elucidating these mechanisms, the present analysis contributes significantly to the nuanced understanding of the pathological underpinnings of WD, thereby providing fresh insights and evidence that may direct innovative therapeutic strategies for this condition.

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Contents

1. Introduction

Copper is a pivotal trace element and a biologically active cofactor within the human body, exerting a critical influence on a multitude of physiological processes, including mitochondrial respiration, biosynthetic pathways and antioxidant defense mechanisms (1). Under physiological steady-state conditions, the body meticulously regulates the equilibrium between copper intake and elimination to preserve optimal copper concentrations. Imbalances characterized by copper insufficiency or excess can precipitate metabolic disorders related to copper, such as Menkes syndrome and Wilson's disease (WD) (2,3). Notably, copper overload is particularly deleterious, as it can lead to the dysregulation of cellular function, ultimately culminating in cell death (4).

The phenomenon of copper-dependent cell death, termed 'cuproptosis', was first described in 2022 (5). This mode of death constitutes a critical node in regulated cell death (RCD), distinguished by its dependence on copper metabolic dynamics and its role in modulating mitochondrial respiration. Cuproptosis differs from other RCD modalities, including apoptosis, pyroptosis, autophagy and ferroptosis (6-8), as it involves the interaction of copper ions with lipoacylated protein components in the tricarboxylic acid (TCA) cycle. These interactions result in the accumulation of lipoacylated proteins and a concomitant reduction in iron-sulfur (Fe-S) cluster proteins, thereby activating a protein-toxicity stress response that culminates in cell death (5).

WD is a genetic disorder of copper metabolism that arises from an autosomal recessive inheritance. Characterized by the excessive accumulation of copper in vital organs, such as the liver, kidneys, brain and cornea, this condition leads to cellular damage, death and a spectrum of organ dysfunction (9). Cuproptosis is intricately linked to the metabolism of copper

ions. The present study aimed to elucidate the molecular underpinnings of cuproptosis and delineate its pivotal role in the pathogenesis of WD, thereby providing a robust scientific foundation and theoretical framework for targeted therapeutic interventions in WD.

2. Copper absorption, transport mechanisms and physiological implications in human biology

The human body primarily acquires copper from dietary and water sources. In adults, the net absorption of copper from the diet is ~1 mg/day. Dietary copper, in conjunction with roughly 4-5 mg of endogenous copper, is secreted into the gastrointestinal tract through diverse digestive juices. The majority of this copper is subsequently returned to the circulation and deposited in various tissues (10). Copper in the human body exists in two primary forms: Cu^{2+} and Cu^{+} (11). At the surface of the small intestinal epithelial cells, dietary Cu^{2+} is reduced to Cu^{+} through the enzymatic actions of prostate metal reductase, six transmembrane epithelial antigen and duodenal cytochrome b. Subsequently, copper ions are absorbed intracellularly via the copper transporter 1(CTR1)/solute carrier family 31 member 1 (SLC31A1) (12-14). During copper transportation, the CTR1 protein effectively maintains copper in its reduced form through two His-Met-Asp cluster structures located at the N-terminus (15-17).

In complex cellular processes, copper ions must be accurately transported to their designated organelles and proteins to prevent harmful effects induced by reactive oxygen species (ROS). Before they arrive at their intended destinations, copper chaperone proteins facilitate the binding of Cu^{+} ions through a sophisticated chaperone mechanism, thereby mitigating oxidative damage that could arise from the production of ROS (18). The pantheon of characterized copper chaperone proteins includes notable entities, such as antioxidant protein 1 (ATOX1), copper chaperone for superoxide dismutase (CCS) and cytochrome c oxidase copper chaperone (COX17). The pivotal role of ATOX1 in facilitating the transport of copper ions to the Golgi apparatus is underscored by its critical function in promoting protein synthesis and activation. Concurrently, CCS plays a vital role in maintaining the intracellular ROS equilibrium by binding copper ions and shuttling them to superoxide dismutase (SOD)1. Additionally, COX17 is predominantly involved in the transport of copper ions from the cytoplasm to the mitochondria, thereby supplying indispensable copper ions to copper-dependent enzymes within this organelle (19,20).

In the regulatory framework governed by the ATPase copper transporting β (ATP7B) protein, a subset of copper ions interacts with α2-globulin, which serves as a precursor to ceruloplasmin, culminating in the formation of this essential copper-containing enzyme (21,22). Concomitantly, as the intracellular copper concentration increases, ATP7A/B proteins play a pivotal role as they are tasked with the efflux of redundant copper ions from the cellular milieu (23,24). As a vital metal-enzyme cofactor, copper is pivotal for numerous physiological processes in the human body. At the cellular level, it facilitates the activity of key enzymes in the respiratory chain, thereby ensuring efficient energy metabolism. In neuroendocrinology, copper is instrumental in the synthesis of neurotransmitters and peptide hormones, thereby enhancing neural signaling and hormonal regulation. Within the context of antioxidant defense mechanisms, copper-dependent enzymes play a critical role in scavenging free radicals, thereby mitigating oxidative damage to cells. Furthermore, in the context of tissue architecture, copper-mediated cross-linking reactions are essential for reinforcing the structural integrity of elastin, collagen and keratin, which underpin the normal form and function of bone, among other tissues. The inherent antimicrobial properties of copper also contribute significantly to its role in immune defense through nutrient provision (25-29). An illustrative diagram delineating the physiological mechanisms underlying copper metabolism is shown in Fig. 1.

3. Cuproptosis: A novel copper-mediated RCD pathway

Characteristics and manifestations of cuproptosis. The phenomenon of 'Cuproptosis' was initially delineated by Tsvetkov *et al* (5), describing it as a distinctive cell death pathway. This mechanism is characterized by its reliance on copper, progressive accumulation of lipoacylated proteins and diminished levels of Fe-S cluster proteins (5). Copper accumulation plays a pivotal role in apoptosis. An increase in the intracellular copper ion concentration triggers a Fenton-like reaction, leading to increased production of ROS and disruption of the intracellular redox balance. Oxidative stress induces lipid peroxidation (30). Concurrently, the impairment of the integrity of the ubiquitin-proteasome system (UPS) results in a significant reduction in proteolytic activity. This interference disrupts the degradation and turnover of cell cycle regulatory proteins and ultimately impedes cell proliferation (31). Furthermore, copper overload severely compromises normal TCA cycle function. Excessive copper promotes the acylation of intracellular TCA enzymes, significantly elevating their acylated forms. Acyl groups directly bind to copper ions, causing the abnormal aggregation of acylated proteins. This process also leads to the loss of Fe-S cluster-containing proteins and induces the production of heat shock protein 70 (HSP70), thereby triggering an acute protein toxicity stress response (32,33). These alterations culminate in mitochondrial dysfunction and cuproptosis (34,35).

The classic morphological manifestations of cuproptosis primarily include mitochondrial contraction, plasma membrane disruption, endoplasmic reticulum (ER) damage and chromatin fragmentation (36). Hu *et al* (37) observed the morphological characteristics of cuproptosis induced by copper nanoparticles and found significant mitochondrial atrophy, reduction or disappearance of cristae and increased membrane density. Li *et al* (38) reported that treatment of HepG2 cells with copper sulfate resulted in prominent ER swelling under a microscope. Zhao *et al* (39) also observed a decrease in inner mitochondrial membrane and ER damage in zebrafish retinal cells treated with copper sulfate. Furthermore, studies have shown that copper induction can damage the membrane structure of chicken liver cells, disrupt the chromatin structure and exacerbate mitochondrial vacuolization (40). Notably, these morphological changes are not unique to cuproptosis and may overlap with or be similar to those of other types of cell death. Therefore, when evaluating the microstructural features associated with cuproptosis, it is

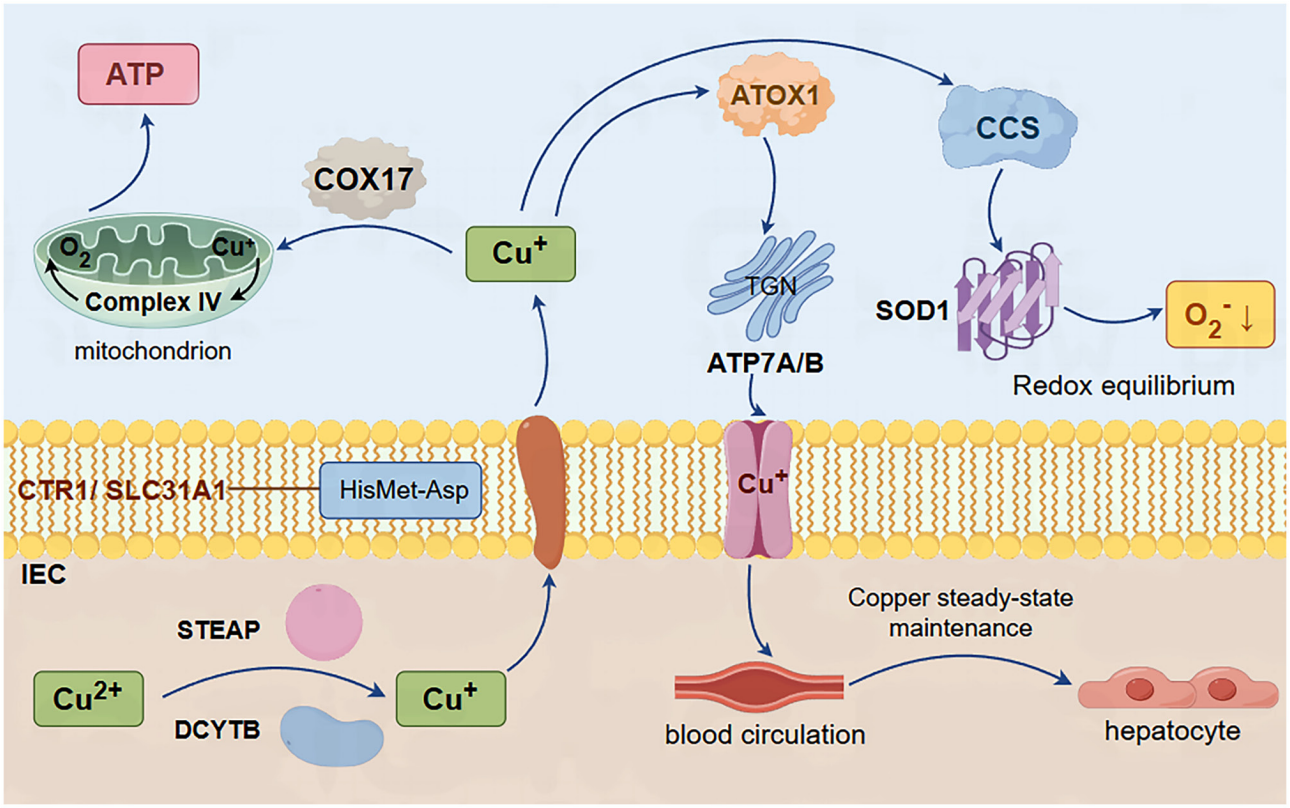

Figure 1. Physiological mechanisms of copper metabolism. ATOX1, antioxidant protein 1; ATP, adenosine triphosphate; ATP7A/B, ATPase copper transporting α/β; IEC, intestinal epithelial cells; CCS, copper chaperone for superoxide dismutase; COX17, cytochrome c oxidase copper chaperone; CTR1, copper transporter 1; SLC31A1; solute carrier family 31 member 1; DCYTB, duodenal cytochrome B; SOD1, superoxide dismutase 1; STEAP, six transmembrane epithelial antigen; TGN, trans Golgi network.

essential to integrate multidimensional molecular biological evidence for a comprehensive assessment.

Molecular mechanisms involved in cuproptosis. The influx and subsequent accumulation of copper within cellular compartments are instrumental in orchestrating the cascade of events that constitute cuproptosis. Central to this mode of cell death is the lipoacylation of proteins, a defining characteristic of cellular proptosis. Notably, ferroxin 1 (FDX1) has emerged as a pivotal upstream regulator in this context (41). Studies have demonstrated that genetic knockout of FDX1 or related acylase genes markedly reduces cuproptosis (42). This finding highlights the potential significance of these genes as critical biomarkers indicative of the progression of cuproptosis. In the realm of cuproptosis-related biomarkers, the current literature identifies a suite of pivotal genes. These include FDX1, lipoyltransferase 1 (LIPT1), lipoic acid synthase (LIAS), dihydrolipoamide dehydrogenase (DLD), dihydrolipoamide S-acetyltransferase (DLAT), pyruvate dehydrogenase E1 subunit α1 (PDHA1), and PDHB. These biomarkers are sentinels, reflecting the intricate metabolic perturbations associated with cuproptosis, and thus merit continued scholarly inquiry and clinical assessment.

FDX1 encodes a small iron-sulfur protein that primarily functions in the electron transport chain, Fe-S cluster biogenesis and regulation of lipoic acid acylation reactions (43). Concurrently, LIPT1 plays a pivotal role in lipid metabolism, primarily by facilitating the transfer of the acyl moiety to the apolipoprotein. This transfer is an important event in the biosynthetic pathway of lipoic acid (44). LIAS, a member of the biotin and lipoic acid synthase family, is predominantly localized in mitochondria. It plays an essential role in catalyzing the biosynthesis of lipoic acid, a crucial molecule involved in various metabolic pathways (45). DLD belongs to the flavin-dependent oxidoreductase class. As a component of the E3 subunit of the pyruvate dehydrogenase complex (PDC), its primary function is to catalyze the dehydrogenation of dihydrolipoamide and convert it into oxidized lipoamide (46). Similarly, DLAT, PDHA1 and PDHB collectively encode key subunits of PDC. As integral components of this complex, they are instrumental in energy metabolism in the human body, facilitating the conversion of pyruvate into acetyl-CoA, which is a critical step in the TCA cycle (47-49).

The integration of whole-genome CRISPR/Cas9 knockout technology has revealed that the knockout of seven pivotal genes, FDX1, LIPT1, LIAS, DLD, DLAT, PDHA1 and PDHB, markedly mitigates the cytotoxic effects associated with copper ion carriers (5). These findings underscore the significance of these genes in the cellular response to copper-induced toxicity. Additionally, several other genes were identified as being implicated in this process, including metal-regulated transcription factor 1 (MTF1), glutaminase (GLS), cyclin-dependent kinase inhibitor 2A, HSP70, dihydrolipoamide branched-chain transacylase E2, SLC31A1, ATOX1, copper chaperone for cytochrome c oxidase 11, dihydrolipoamide S-succinyltransferase and transporters of copper

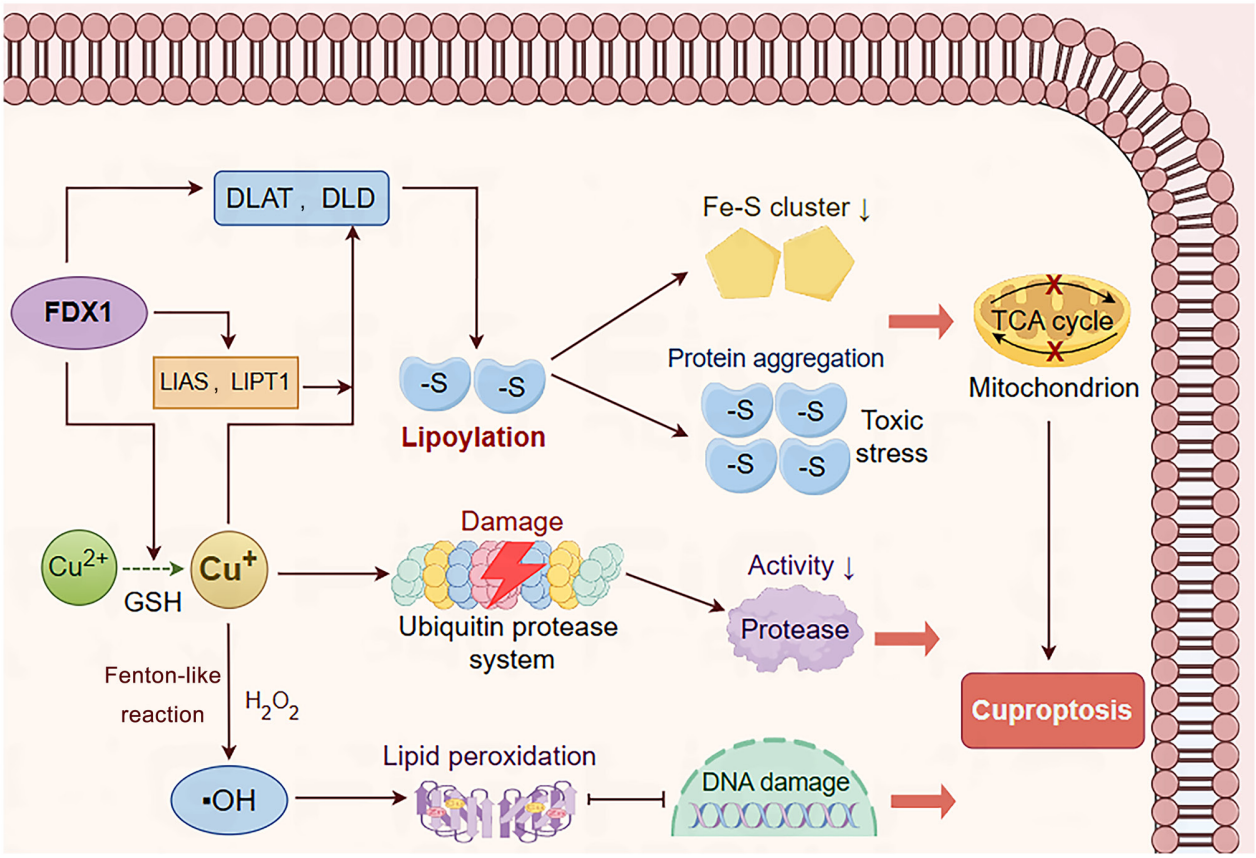

Figure 2. Schematic representation of the pathological process in cuproptosis. DLAT, dihydrolipoamide S-acetyltransferase; DLD, dihydrolipoamide dehydrogenase; FDX1, ferroxin 1; GSH, glutathione; LIAS, lipoic acid synthase; LIPT1, lipoyltransferase 1; TCA, tricarboxylic acid.

and zinc 1 (ZnT1) (50-54). This comprehensive gene network highlights the intricate molecular mechanisms underlying cellular responses to copper ion exposure.

In addition, the tumor suppressor p53 plays a crucial role in metabolic regulation. This factor not only inhibits the activity of glycolysis but also promotes oxidative phosphorylation in the metabolic pathway of cancer cells. Notably, p53 plays a significant role in the regulation of glutathione (GSH) production. By controlling the expression of genes involved in Fe-S cluster biosynthesis, p53 may play an important role in the mechanism of cuproptosis. Previous studies have found that FDXR (FDX1/2) plays a key role in iron metabolism under p53 regulation and may be related to the maintenance of Fe-S cluster stability (55). In addition, p53 can activate the expression of the iron-sulfur cluster assembly enzyme and frataxin genes, which are primarily responsible for synthesizing scaffold proteins crucial for the iron-sulfur cluster assembly process (56-58). Consequently, p53 likely plays a pivotal role in regulating cuproptosis mediated by FDX1.

Overall, for the key genes associated with cuproptosis that have been identified thus far, copper metabolism can be regulated through the following mechanisms, thereby influencing cuproptosis: i) Regulation of copper ion uptake; for example, SLC31A1 affects copper metabolism by modulating the cellular uptake of copper ions (59). ii) Involvement in the transport and storage of copper ions: For instance, ATOX1 regulates copper ion levels by binding and transporting them (60). iii) Modulation of metabolic pathways related to copper ions, such as FDX1, regulates copper metabolism by affecting key proteins in the mitochondrial respiratory chain (61). Further studies are required to elucidate the underlying mechanisms. A mechanistic diagram based on the pathological process of cuproptosis is shown in Fig. 2.

4. Role of cuproptosis in the pathogenesis of WD

The pathogenesis of WD. WD arises from mutations in the ATP7B gene and was first reported by Kinnier Wilson *et al* (62). ATP7B, which is located on chromosome 13, spans 21 exons and produces a transcript of ~7.5 kilobases in length. This transcript encodes a substantial transmembrane protein consisting of 1,465 amino acids. ATP7B is predominantly expressed in the liver, kidneys and placenta (63-65). It plays a crucial role in intracellular copper metabolism in hepatocytes by facilitating the transport of copper ions across the cellular membrane into the Golgi apparatus. Subsequently, this copper is directed towards lysosomes and is eventually excreted into the bile, thereby maintaining homeostasis and preventing copper overload. Additionally, in the Golgi apparatus, ATP7B protein is essential for ceruloplasmin synthesis. This process ultimately facilitates the secretion of copper-bound ceruloplasmin into the circulatory system (66,67).

Mutations in ATP7B cause concomitant dysfunction of the protein, which in turn diminishes its capacity to transport

copper ions effectively. This decrease in transport efficacy manifests as diminished synthesis of ceruloplasmin within the serum and a compromised ability to excrete copper through the biliary ducts. Consequently, there is a tendency for copper to accumulate excessively within the liver or be released into the circulatory system, where it may deposit in various organs, including the brain, kidneys and corneas (68). This pathological accumulation is capable of precipitating a spectrum of clinical symptoms, including liver damage, neurological abnormalities, cardiac and renal impairments, development of Kayser-Fleischer rings in the cornea and hemolytic anemia (69).

In WD, liver injury may manifest with various clinical presentations, such as acute hepatitis, liver fibrosis and cirrhosis. It has been reported that these morbid conditions are intricately linked to the hepatic stellate cell activation induced by copper deposition. This activation is further associated with the accumulation of extracellular matrix components and proliferation of myofibroblasts, ultimately contributing to the degeneration and necrosis of hepatocytes (70-73). The clinical manifestations of neurological impairment may include a spectrum of symptoms, such as tremors, ataxia, dysphagia, alterations in emotional regulation and significant memory deficits. Previous studies have shown that WD-related neuropathy predominantly affects the basal ganglia, thalamus, brainstem and cerebellum. Pathological examination revealed axonal swelling, spheroid formation and demyelination (74-78). Clinically, when WD is combined with renal involvement, the symptoms include hematuria, proteinuria and edema of the face and lower extremities. Studies indicate that renal tubular epithelial injury is a defining feature characterized by cytoplasmic vacuolization, increased cell volume and nuclear fragmentation (69,79,80). The presence of a Kayser-Fleischer ring and corneal copper deposition correlates with ocular symptoms (81,82). Furthermore, patients with WD who also experience cardiac injury may present with atrial fibrillation, heart failure and autonomic dysfunction. These symptoms have been attributed to copper deposition-induced myocardial fibrosis, small vessel sclerosis and inflammatory cell infiltration (83-85). In addition, reproductive dysfunction caused by WD is primarily characterized by hypogonadism and teratozoospermia, which are linked to mechanisms such as inflammation, pyroptosis and apoptosis triggered by copper deposition (86-88). WD can also affect the skeletal system, leading to conditions such as osteomalacia and osteoporosis, which may be associated with secondary renal dysfunction (89,90). Additionally, WD may cause blood system-related symptoms, such as hemolytic anemia, potentially due to copper-induced hemoglobin oxidation reactions (91-93). The corresponding clinical manifestations and pathological mechanisms are summarized in Table I.

The key role of cuproptosis in WD

Activation of oxidative stress and impairment of the antioxidant defense system. The activation of oxidative stress can induce apoptosis. In WD, increased copper concentrations promote the formation of high levels of ROS through Fenton-like reactions (94) or by inhibiting the activity of mitochondrial respiratory chain complexes (95). Excessive ROS directly trigger oxidative stress by attacking cellular lipids, proteins and DNA, thereby causing cellular damage. When this damage reaches a certain threshold, apoptosis is induced (96). Nuclear factor κB (NF-κB) stands as a pivotal regulator of cell survival, modulated by ROS. Previous research has demonstrated that copper can elevate ROS levels in BV2 cells (mouse microglia), thereby activating the NF-κB pathway. This activation subsequently leads to a reduction in the mitochondrial membrane potential and decreased expression of Parkin and phosphatase and tensin homolog-induced kinase 1, ultimately resulting in cell death (97). Furthermore, the synergistic action of disulfiram and Cu^{2+} enhanced ROS production and activated the p38 mitogen-activated protein kinase (MAPK) signaling pathway. Concurrently, the NF-κB signaling pathway is repressed, ultimately resulting in breast cancer cells (98). Hence, the deposition of copper in WD can elicit cell death through oxidative stress-related signaling pathways, a phenomenon intimately associated with cuproptosis.

Furthermore, impairment of the antioxidant defense system is closely associated with the induction of cuproptosis. In WD, excessive intracellular copper accumulation inhibits the activity of key antioxidant enzymes, including SOD, thereby compromising the cellular capacity to neutralize ROS (99). When the antioxidant defense system fails to effectively eliminate ROS, these oxidants accumulate precipitously (100), leading to elevated intracellular oxidative stress, which subsequently triggers cuproptosis. Additionally, copper overload depletes critical intracellular antioxidants, such as GSH. As a principal nonenzymatic antioxidant, GSH normally scavenges ROS through conversion to oxidized GSH (101,102). However, excess copper directly binds to GSH, causing its rapid consumption (103) and significantly diminishing the cellular antioxidant reserves. This compromised defense mechanism renders the cells vulnerable to ROS-mediated damage, ultimately driving cuproptosis. Thus, the interplay between oxidative stress and antioxidant system dysfunction synergistically promotes the progression of cuproptosis in WD, representing a pivotal pathogenic mechanism in this disease.

Dysfunction of the UPS. UPS constitutes a refined mechanism for selective protein hydrolysis by dismantling ubiquitin-conjugated substrates via proteasomal degradation (104). The initiation of this system is contingent upon activation by the E1 ubiquitin-activating enzyme, which subsequently facilitates the recognition and linkage process through the binding of E2 conjugating enzymes. This intricate cascade is further orchestrated by E3 ligases, ultimately culminating in the proteolytic degradation and subsequent recycling of targeted proteins (105,106). The UPS plays a pivotal role in the maintenance of protein homeostasis, regulation of the cell cycle and modulation of diverse signaling pathways (107-109). In WD, copper ions selectively bind to specific subunits of the constitutive proteasome, thereby diminishing its activity. This interference disrupts the intracellular redox equilibrium, thereby disturbing the efficiency and progression of the ubiquitination cascade. The resultant abnormal accumulation of copper ions may precipitate the misfolding or aggregation of critical proteins, resulting in the formation of protein aggregates. Such aggregates frequently evade degradation by the ubiquitin-proteasome system, thereby augmenting cellular stress and culminating in the functional impairment of cells (31).

Table I. Summary of clinical features and pathological mechanisms of WD.

Organs affected by WD	Clinical features	Pathological mechanism	(Refs.)
Liver	Fatigue, decreased appetite, bloating; Abnormal liver function, jaundice and coagulation dysfunction; Splenomegaly, esophageal and gastric varices, ascites	Copper deposition induces activation of hepatic stellate cells, accumulation of extracellular matrix, proliferation of myofibroblasts, degeneration and necrosis of liver cells	(70-73)
Brain	Tremors, dystonia, swallowing difficulties; Ataxia; Emotional changes, memory decline	The lesion is mainly located in the basal ganglia area, including the thalamus, brainstem and cerebellum, with pathological manifestations such as axonal swelling, spheroid formation and demyelination of nerve cells	(74-78)
Kidneys	Hematuria, proteinuria, facial and lower limb edema	Mainly characterized by damage to renal tubular epithelial cells, pathological manifestations include vacuolization, volume increase and nuclear rupture	(69,79,80)
Eyes	Kayser-Fleischer ring	Copper deposition in the cornea	(81,82)
Heart	Atrial fibrillation, heart failure, autonomic dysfunction	Myocardial fibrosis, small vessel sclerosis and inflammatory cell infiltration induced by copper deposition	(83-85)
Reproduction	Hypogonadism, teratozoospermia	Related to mechanisms such as inflammatory response, pyroptosis and apoptosis caused by copper deposition	(87,88)
Bones	Osteomalacia, osteoporosis	Related to renal insufficiency	(89,90)
Hemocyte	Hemolytic anemia	Hemoglobin oxidation caused by copper	(91-93)

WD, Wilson's disease.

Studies have shown that copper complexes exert a profound inhibitory effect on proteasome activity in human cancer cells (110). Furthermore, the dithioamine-Cu^{2+} complex has been demonstrated to potently inhibit the degradation of ubiquitinated proteins by obstructing upstream signaling pathways of the protease system and suppressing ubiquitination-dependent ATP synthase activity (111). Collectively, these findings suggest that the accumulation of copper in WD may perturb cellular copper homeostasis, thereby affecting cell death via the ubiquitin-proteasome system and its associated signaling pathways.

Mechanisms of cuproptosis induced by specific protein toxicity and associated stress responses. Firstly, excess copper ions can bind to specific proteins containing particular amino acid motifs, thereby disrupting their normal functions. In the TCA cycle, lipid acylation, triggered by the deposition of WD copper ions, is a critical step in initiating cell death (112). This reaction transfers lipid acyl groups to specific amino acid residues of enzymes, resulting in the lipid acylation modification of TCA enzymes, which affects their activity and function (113). Additionally, copper overload can lead to protein misfolding and aggregation aberrantly interacting with proteins, thereby interfering with normal cellular physiological functions and ultimately causing toxic damage to cells. Abnormal elevations in copper ion concentrations can cause abnormal aggregation

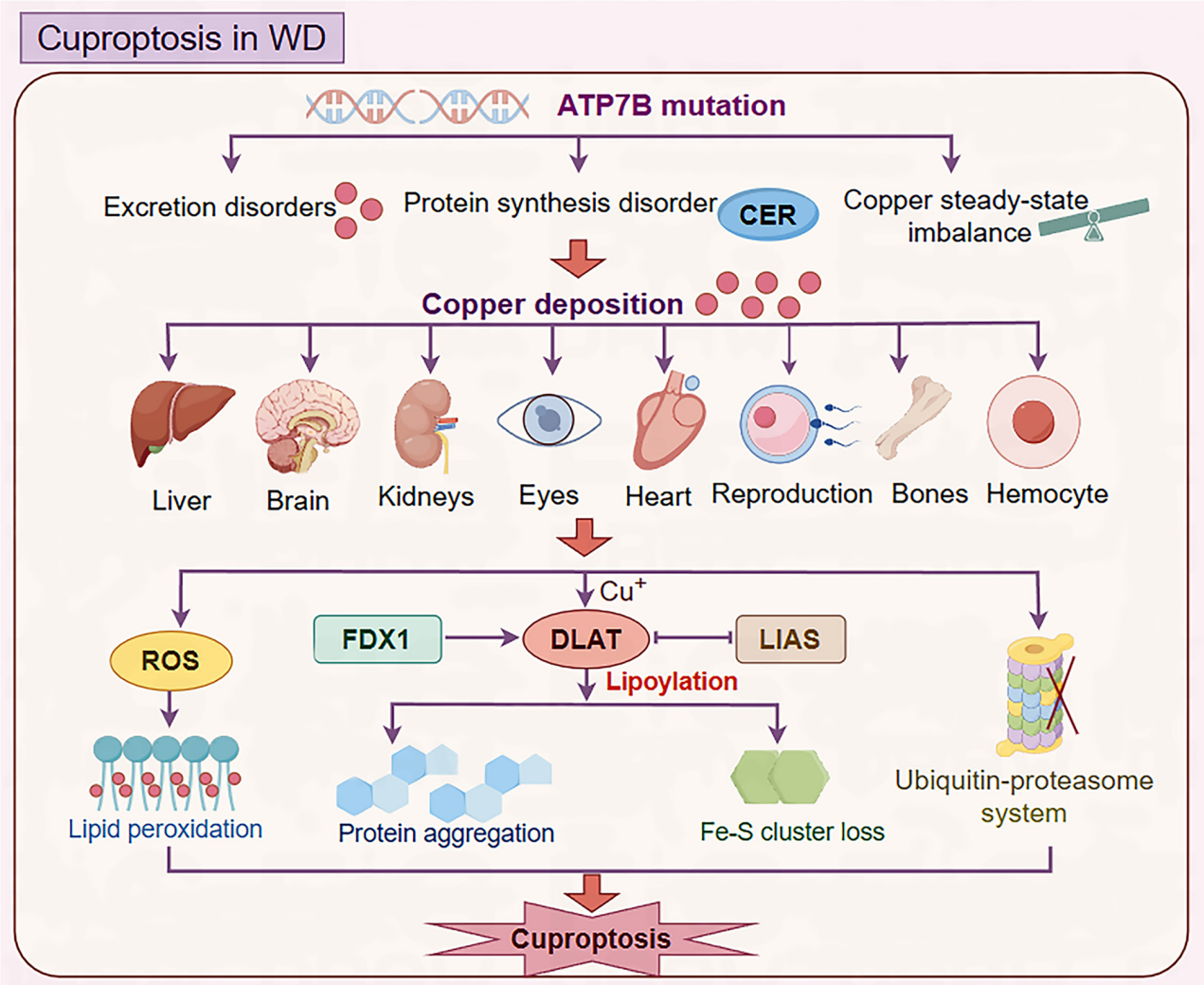

Figure 3. Mechanistic diagram of cuproptosis induced by WD. WD, Wilson's disease; ATP7B, ATPase copper transporting β; CER, ceruloplasmin; DLAT, dihydrolipoamide S-acetyltransferase; FDX1, ferroxin 1; LIAS, lipoic acid synthase; ROS, reactive oxygen species.

of lipid-acylated proteins, subsequently impairing the normal function of Fe-S cluster proteins in the mitochondrial respiratory chain (114).

Notably, when intracellular protein aggregation and loss of Fe-S cluster proteins occur, they trigger a series of acute stress responses, such as the unfolded protein response (UPR) and heat shock response. Initially, these abnormal signals are perceived by intracellular stress sensors, initiating UPR (115). The UPR inhibits protein synthesis, reduces the production of new misfolded proteins and regulates gene expression related to protein folding and ER-associated degradation, thereby enhancing the ability of cells to handle misfolded proteins and maintain intracellular proteostasis (116). Furthermore, when cells are subjected to stress stimuli associated with cellular proptosis, they activate heat shock factor 1 (117), thereby promoting the expression of heat shock proteins, such as HSP70 (118). HSP70 recognizes and binds to misfolded or aggregated proteins, assisting them in refolding or transporting them to the proteasome for degradation, thereby mitigating the detrimental effects of protein toxicity in cells (119). The underlying mechanism of WD-induced cuproptosis is shown in Fig. 3.

Potential association between genes involved in cuproptosis and WD. Studies have reported that numerous genes co-expressed with FDX1 play pivotal roles in various mitochondrial respiratory metabolic activities. Furthermore, these genes are associated with the Notch signaling pathway (42). Our group previously identified the enrichment of key signaling pathways, including Notch and MAPK, through differential expression analysis of long non-coding RNAs in a toxic milk (TX) mouse model, which is recognized as an ideal animal model for WD research (120). Additionally, research in immunology has revealed close associations between FDX1 and several immune-related pathways, such as inflammatory responses and the TNF-α/NF-κB signaling pathway (121). It has been confirmed that patients with WD exhibiting liver and nervous system damage show significant elevations in plasma levels of type 1 T-helper (Th1) cells (TNF-α and TNF-β), Th3 (TGF-β1) and Th17 (IL-23) (122). Furthermore, recent investigations have elucidated that the accumulation of copper ions activates the Toll-like receptor 4/NF-κB signaling cascade. This activation was associated with a marked increase in the levels of inflammatory cytokines in the serum and testicular compartments

of TX mice (123). Collectively, these findings suggest that FDX1, a pivotal regulatory gene, may serve as a critical determinant of the cuproptosis in WD.

LIPT1 is a pivotal regulator of the lipoic acid metabolic cascade and plays a crucial role in the orchestration of mitochondrial energy metabolism (124,125). Recent investigations have shown that the hepatic pathology observed in patients with WD, as well as in the corresponding animal models, is characterized by varying degrees of mitochondrial copper accumulation. This excess copper not only precipitates the disintegration of mitochondrial integrity but also leads to the structural degradation of mitochondrial components (126). Collectively, these findings suggest that LIPT1 may serve as a critical modulator of the copper-mediated pathophysiology of WD by influencing the TCA cycle and intricate mechanisms of mitochondrial energy metabolism.

Previous studies have established a compelling association between LIAS and the pathophysiological mechanisms underlying oxidative stress and inflammatory responses. In a murine model of diabetic nephropathy, LIAS was identified as a pivotal factor for preserving mitochondrial integrity through its regulatory influence on the expression of inflammatory mediators. This regulatory function is involved in oxidative stress responses, encompassing signaling cascades, such as the MAPK pathway, and the promotion of antioxidant mechanisms, including the nuclear factor erythroid 2-related factor 2 (NRF2) signaling pathway (127). Our research group previously corroborated the significance of the MAPK signaling pathway in the etiology of WD (120). Furthermore, a recent study demonstrated that Nrf2 expression is markedly elevated in PC12 cells following copper loading in a WD neural injury model (128). This finding suggests a potential link between LIAS and copper-mediated pathogenesis of WD, underscoring the intricate interplay between these molecular pathways in disease progression.

DLD and DLAT, which are integral components of the PDC, are strongly associated with glucose metabolism. Inhibition of DLD enzymatic activity has been shown to effectively decelerate the glycolytic pathway of glucose, thereby significantly diminishing its metabolic rate (129,130). Using proton nuclear magnetic resonance metabolomics, our research team observed that the liver concentrations of glycogen, α glucose and lactate in TX mice were notably elevated compared to those in the control group (131). These findings indicate the potential roles of DLD and DLAT in WD pathogenesis.

PDHA1 and PDHB, integral subtypes of PDC, are pivotal in the glycolytic and TCA cycle metabolic pathways (132,133). Concurrently, extracellular signal-regulated kinase (ERK), a key member of the MAPK family, plays a critical role in cellular signaling transduction. It has been demonstrated that fisetin-induced inhibition of ERK-1/2 phosphorylation triggered by fisetin can significantly suppress the expression of PDHA1, thereby mitigating cellular apoptosis (134). Furthermore, cell proliferation and migration, which are modulated by the PDHB and ERK signaling pathways, are intricately interconnected (135). Wang *et al* (136) reported that the deposition of copper in WD may perturb the secretion of reproductive hormones by inducing apoptosis in hypothalamic-pituitary cells and suppressing ERK signaling in mice. This disruption resulted in diminished male fertility in mice. Collectively, these findings suggested that PDHA1 and PDHB may serve as potential biomarkers of copper-dependent toxicity, indicating their association with WD to a certain extent.

GLS, a pivotal enzyme in the catabolic pathway of glutamine, has emerged as a critical regulatory protein in the mechanism of cuproptosis (137). Glutamine, a copious nonessential amino acid in the cellular environment, plays a fundamental role in diverse biological processes, including protein synthesis, immune modulation and cellular metabolism (138). Studies have revealed altered metabolism in hepatic glutamine levels in TX mice compared with those in the normal group (131), suggesting that GLS may influence the body's redox imbalance by modulating protein and energy metabolism (139), which is implicated in WD-associated cuproptosis.

p53 plays a pivotal role in WD-related cuproptosis, potentially through its regulatory effects on copper ion homeostasis and the oxidative defense system. It has been shown that p53 modulates cellular copper uptake by regulating the expression of the copper transporter CTR1, thereby altering copper ion stability and sensitivity to cuproptosis (140). Furthermore, p53 activates the transcription of antioxidant genes, including SOD2 and catalase, thereby enhancing ROS scavenging and mitigating oxidative stress damage (141). Notably, a substantial increase in p53 mutations has been observed in liver samples from patients with WD (142), highlighting the multifaceted involvement of p53 in the pathogenesis of cuproptosis in WD.

Additionally, research has identified several metal homeostasis regulators, such as MTF1 and ZnT1, that participate in WD-related copper toxicity by interacting with copper ions or competitively binding to shared sites, thereby influencing intracellular copper concentrations (53,143).

In summary, the currently identified cuproptosis-related genes may modulate cuproptosis through the following core mechanisms in WD: i) Regulation of lipoic acid metabolism: Genes such as FDX1, LIPT1 and LIAS can influence the TCA cycle and Fe-S cluster stability by participating in lipoic acid metabolism; ii) modulation of energy metabolism: Genes like DLD, DLAT, PDHA1/PDHB regulate the coupling of glycolysis to the mitochondrial respiratory chain, leading to ATP synthesis impairment and lactic acidosis; iii) control of oxidative stress and inflammation: Genes including p53 and GLS may trigger lipid peroxidation and the release of inflammatory cytokines by regulating oxidative stress; and iv) metal homeostasis regulation: Genes such as MTF1 and ZnT1 indirectly affect cuproptosis by adjusting metal homeostasis or competing ion transport. The associations between copper death-related genes and WD are summarized in Table II.

The present study had several limitations. First, as a recently discovered form of cell death, the core molecular machinery of cuproptosis remains incompletely elucidated, particularly regarding the coordinated actions of the key molecular players that trigger cuproptosis in WD, an area that warrants more systematic investigation. Second, although the present study identified and characterized the pivotal role of cuproptosis in the pathogenesis of WD, the specific molecular mechanisms

Table II. Relationship between partial cuproptosis-related genes and WD.

Gene name	Function	Bridge between genes and WD	Relationship between genes and WD	(Refs.)
FDX1	Encodes iron sulfur protein, involved in electron transfer chain and Fe-S cluster biosynthesis; regulation of the esterification reaction of lipoic acid	Associated with FDX1 co-expression, immune- and inflammation-related genes	Involved in key signaling pathways such as Notch and NF-κB, as well as the expression of immune and inflammatory factors, including TNF-α, TNF-β, TGF-β1 and IL-23	(42,120-123)
LIPT1	Participates in the biosynthesis of lipoic acid	Regulation of mitochondrial energy metabolism	Mitochondrial copper overload, breakdown of mitochondrial membrane and structural damage	(124-126)
LIAS	Catalysis in the biosynthesis of α-lipoic acid	Oxidative stress, inflammatory response	Regulation of the MAPK signaling pathway and NRF2 gene expression	(127,128)
DLD, DLAT	E3 constituent of PDC; E2 constituent of PDC	Glucose metabolism	Affecting the levels of glycogen, α glucose and lactate	(129-131)
PDHA1, PDHB	E1 constituent of PDC	Cell signaling transduction	Regulation of the ERK signaling pathway	(132-136)
GLS	Catalyzes the hydrolysis of glutamine into glutamic acid and ammonia	Protein, energy metabolism and redox balance	Affecting glutamine levels	(131,137-139)
p53	Regulates glutathione production and participates in gene expression related to Fe-S cluster biosynthesis	Regulates the steady-state of copper ions and the oxidation defense system	Regulates CTR1 and antioxidant gene expression	(140-142)
MTF1	Maintains the steady state of metal ions	Interacts with copper ions	Promotes the expression of genes such as metallothionein 1X	(143)
ZnT1	Zinc transporter, a novel copper transporter	Affects copper uptake	Zn^{2+} competes with Cu^{2+} for the ZnT1 binding site, affecting copper metabolism	(53)

WD, Wilson's disease; CTR1, copper transporter 1; DLAT, dihydrolipoamide S-acetyltransferase; DLD, dihydrolipoamide dehydrogenase; ERK, extracellular regulated protein kinases; FDX1, ferroxin 1; GLS, glutaminase; IL-23, interleukin-23; LIAS, lipoic acid synthase; LIPT1, lipoyltransferase 1; MAPK, mitogen-activated protein kinase; MTF1, metal-regulated transcription factor 1; NF-κB, nuclear factor κB; NRF2, nuclear factor erythroid 2-related factor 2; PDC, pyruvate dehydrogenase complex; p53, tumor protein 53; PDHA1, pyruvate dehydrogenase E1 subunit α1; PDHB, pyruvate dehydrogenase E1 subunit β; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α; ZnT1, transporters of copper and zinc 1.

require further experimental investigation and validation. Third, although therapeutic strategies targeting cuproptosis hold translational promise, clinical implementation faces significant challenges, including the precise modulation of cuproptotic pathways without disrupting physiological copper metabolism and overcoming tissue-specific barriers

in drug delivery. Future studies should integrate multi-omics approaches, organoid modeling and clinical cohort analyses to comprehensively decipher cuproptotic molecular networks and facilitate the development of novel mechanism-based therapeutic interventions.

5. Conclusions

Copper, an indispensable trace element and a pivotal cofactor within the human body, plays a critical role in myriad physiological processes. Disturbances in copper homeostasis and deposition, as observed in WD, significantly contribute to cellular dysregulation. This dysregulation orchestrates a symphony of cellular responses, including activation of oxidative stress, impairment of the ubiquitin-proteasome system and activation of protein toxicity stress responses, all of which converge to regulate cell death. Lipoacylation of proteins is a crucial step in the cuproptosis observed in WD. FDX1 has emerged as a key regulatory node in this intricate network, exerting a profound influence on cuproptosis. It serves as a sentinel with landmark significance, orchestrating the expression of pivotal marker proteins, such as LIPT1, LIAS, DLD, DLAT, PDHA1 and PDHB. This study systematically delineates the molecular underpinnings of cuproptosis and provides a comprehensive overview of the mechanisms underlying its pivotal role in the pathogenesis of WD. By integrating critical molecular clusters, the present review provided a comprehensive framework for understanding the coordinated regulation of cuproptosis in WD. Concomitantly, targeted therapies directed at this mechanism hold promise as novel and prospective treatment approaches, thereby offering more precise and effective therapeutic strategies for patients with WD. Consequently, this could lead to a significant enhancement in quality of life and survival rates.

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Availability of data and materials

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Authors' contributions

HC and XW contributed to the design of the study and writing of the manuscript. JX and YP performed the literature research. HY and YM wrote the main manuscript text and prepared figures. JZ revised the article critically for important intellectual content and provided final approval of the version to be submitted. All authors reviewed the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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