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Copper Metabolism and Cuproptosis: Molecular Mechanisms and Therapeutic Perspectives in Neurodegenerative Diseases*

Xiao-xia BAN[1], Hao WAN[1], Xin-xing WAN[2], Ya-ting TAN[1], Xi-min HU[3], Hong-xia BAN[4], Xin-yu CHEN[1], Kun HUANG[1], Qi ZHANG[1, 5#], Kun XIONG[1, 5, 6#]

[1]*Department of Human Anatomy and Neurobiology, School of Basic Medical Science, Central South University, Changsha 430013, China*
[2]*Department of Endocrinology, Third Xiangya Hospital, Central South University, Changsha 430013, China*
[3]*Department of Dermatology, Xiangya Hospital, Central South University, Changsha 430013, China*
[4]*Affiliated Hospital, Inner Mongolia Medical University, Hohhot 010050, China*
[5]*Key Laboratory of Emergency and Trauma of Ministry of Education, Hainan Medical University, Haikou 571199, China*
[6]*Hunan Key Laboratory of Ophthalmology, Changsha 430013, China*

[Abstract] Copper is an essential trace element, and plays a vital role in numerous physiological processes within the human body. During normal metabolism, the human body maintains copper homeostasis. Copper deficiency or excess can adversely affect cellular function. Therefore, copper homeostasis is stringently regulated. Recent studies suggest that copper can trigger a specific form of cell death, namely, cuproptosis, which is triggered by excessive levels of intracellular copper. Cuproptosis induces the aggregation of mitochondrial lipoylated proteins, and the loss of iron-sulfur cluster proteins. In neurodegenerative diseases, the pathogenesis and progression of neurological disorders are linked to copper homeostasis. This review summarizes the advances in copper homeostasis and cuproptosis in the nervous system and neurodegenerative diseases. This offers research perspectives that provide new insights into the targeted treatment of neurodegenerative diseases based on cuproptosis.

Key words: cuproptosis; copper metabolism; copper homeostasis; neurodegeneration; neurodegenerative disease

Copper was first detected in animal tissues more than 100 years ago[1]. Copper participates in significant physiological processes, such as energy metabolism, mitochondrial respiration, enzyme synthesis, and oxidative stress[2]. Copper serves as a safeguard for human health. Typically, the body only absorbs a minute amount of copper to maintain a stable and appropriate level within cells. The disruption of copper homeostasis can elevate copper levels, which in turn, leads to copper toxicity and cell death, while copper deficiency can lead to diseases[3]. Thus, both systemic and cellular copper metabolism are tightly regulated. Abnormalities in copper homeostasis can be caused by genetic mutations, cellular senescence, or environmental factors, leading to cancer, inflammation, and neurodegenerative diseases[4].

Cell death is an essential process in organism development[5–8]. The different forms of cell death can be classified into regulatory and non-regulatory types[9–13], such as apoptosis, necroptosis, and ferroptosis. Apoptosis is a classical cell death that results in the removal of injured cells to maintain normal physiological activities. Apoptotic cells can form apoptosis bodies, which are subsequently phagocytosed by macrophages[14]. Necroptosis is also regulated, but it is distinct from apoptosis, which contributes to defending against viral and bacterial infections, multiorgan inflammations, and so on[15]. Ferroptosis is an iron-dependent and non-apoptotic cell death that is caused by redox imbalance. Ferroptosis mainly occurs through transporter-dependent and enzyme-regulated pathways[16]. Cuproptosis is a recently discovered phenomenon caused by the excess of copper ions. It occurs predominantly within the mitochondria, where copper binds to lipoylated proteins of the tricarboxylic acid (TCA) cycle, leading to protein aggregation, loss

Xiao-xia BAN, E-mail: xxb19982021@163.com
#Corresponding authors, Kun XIONG, E-mail: xiongkun2001@163.com; Qi ZHANG, E-mail: zhangqi2014@csu.edu.cn
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of iron-sulfur cluster proteins, proteotoxic stress, and ultimately, cell death[17]. The discovery of cuproptosis has provided new opportunities for the investigation of systemic illnesses and injuries.

The physiological state and function of neurons are regulated by copper[4]. An imbalance in copper homeostasis can lead to neuronal damage, or even degenerative lesions[18]. In addition, astrocytes and microglia are crucial for the regulation of copper homeostasis within the brain[19]. Various therapeutic approaches have been utilized to treat conditions associated to disrupted copper homeostasis in the brain, including major neurodegenerative disorders[20]. Cuproptosis contributes to our understanding of disease development, and the management of neurodegenerative diseases. The present study provides an overview of the functions and mechanisms of copper and cuproptosis in the nervous system. In addition, the potential for future research and therapy based on copper homeostasis and cuproptosis in the nervous system and neurodegenerative disorders was discussed.

1 SYSTEMIC AND CELLULAR COPPER METABOLISM IN THE HUMAN BODY

1.1 Systemic Copper Metabolism in the Human Body

Copper is a crucial component of at least 20 enzymes distributed in various vital tissues[21]. In 1928, Hart reported that anemia in rats can only be resolved *via* supplementation with copper combined with iron[22]. In the normal human body, 50%–70% of copper is located in the muscle and bone, 20% of copper is located in the liver, and the remaining 5%–10% of copper is located in the blood[23]. Copper is mainly absorbed in the small intestines, and is mediated by copper transporter protein 1 (CTR1, also known as SLC31A1)[24]. Copper is converted from Cu^{2+} to Cu^{+} by metal reductases, such as duodenal cytochrome b and six-transmembrane epithelial antigen of the prostate for absorption[25]. By studying patients with Wilson disease characterized by copper-overload ATP7B deficiency, researchers have discovered that the decrease in transcriptional activity and expression of CTR1 may represent an adaptive cellular regulation mechanism in response to copper disruption. This mechanism prevents excessive copper from entering the small intestinal cells[26]. Divalent metal transporter 1 (DMT1) also transports divalent copper ions[27]. Copper is transported through the basolateral membrane of small intestinal epithelial cells *via* the ATP7A protein, and subsequently into the portal circulation, where this ultimately reaches the liver[28]. ATP7A is predominantly found in the basolateral membrane. It plays a crucial role in Menkes disease, which is characterized by copper deficiency resulting from genetic abnormalities in ATP7A. Defective copper transportation in small intestinal epithelial cells results in abnormal copper metabolism[29].

Regardless of the level of copper in the body, a number of regulatory mechanisms are activated to maintain normal copper metabolism. Transcription factor specificity protein 1 (Sp1) may regulate copper metabolism. Sp1 binds to GC boxes located at the CTR1 promoter, and its levels are regulated by high and low levels of copper. Elevated copper levels inhibit the binding of Sp1 to the CTR1 and Sp1 promoters[30]. Metal-binding transcription factor (MTF1) is correlated to the regulation of copper homeostasis. Excess copper leads to the transcriptional activation of metallothionein and the nuclear expression of MTF1, resulting in enhanced metallothionein levels. Conversely, when copper is deficient, MTF1 promotes CTR1B transcription and expression, resulting in increased copper uptake, and the maintenance of copper homeostasis[31]. Copper ions do not freely circulate in the bloodstream. Instead, approximately 85%–95% of copper ions bind to ceruloplasmin to form complexes, which cannot be exchanged. Copper ion exchange occurs when these complexes reach the organs and tissues, such as the heart, liver, brain, kidneys, intestines, lungs, and spleen[32, 33] (fig. 1).

The liver is the primary organ in the body for copper storage. The complex and highly organized regulation of copper metabolism occurs within hepatocytes[34, 35]. Copper is secreted in an ATP7B-dependent manner for vesicular excretion, or bound to ceruloplasmin for release into the circulation, in order to reach other organs and tissues. In the liver, the primary pathway for eliminating endogenous copper is mediated *via* the secretion of vesicles produced by the Golgi apparatus, which transport copper for export to the bile *via* ATP7B, followed by excretion in feces[36]. Neurons release copper at the synapse for encapsulation in vesicles. Synaptic vesicles contain high levels of copper. Following cellular depolarization, copper is released into the synaptic gap to modulate various membrane receptor functions[37].

1.2 Cellular Copper Metabolism in the Human Body

Hepatocytes transfer copper into cells *via* CTR1[38]. Intracellular copper transportation to primary targets is effectively regulated by copper chaperone proteins within the cytoplasm (fig. 2).

1.2.1 Chelation Glutathione binds copper for detoxification. This scavenges free radicals, and binds to heavy metal ions, such as mercury, cadmium, and arsenic, for excretion[39]. Copper is chelated by metallothionein1/2, which binds copper ions *via* cysteine residues in a pH-dependent manner[40].

1.2.2 COX17 Copper chaperone COX17 binds copper ions in the space between the mitochondrial membranes. Copper is crucial for the synthesis of

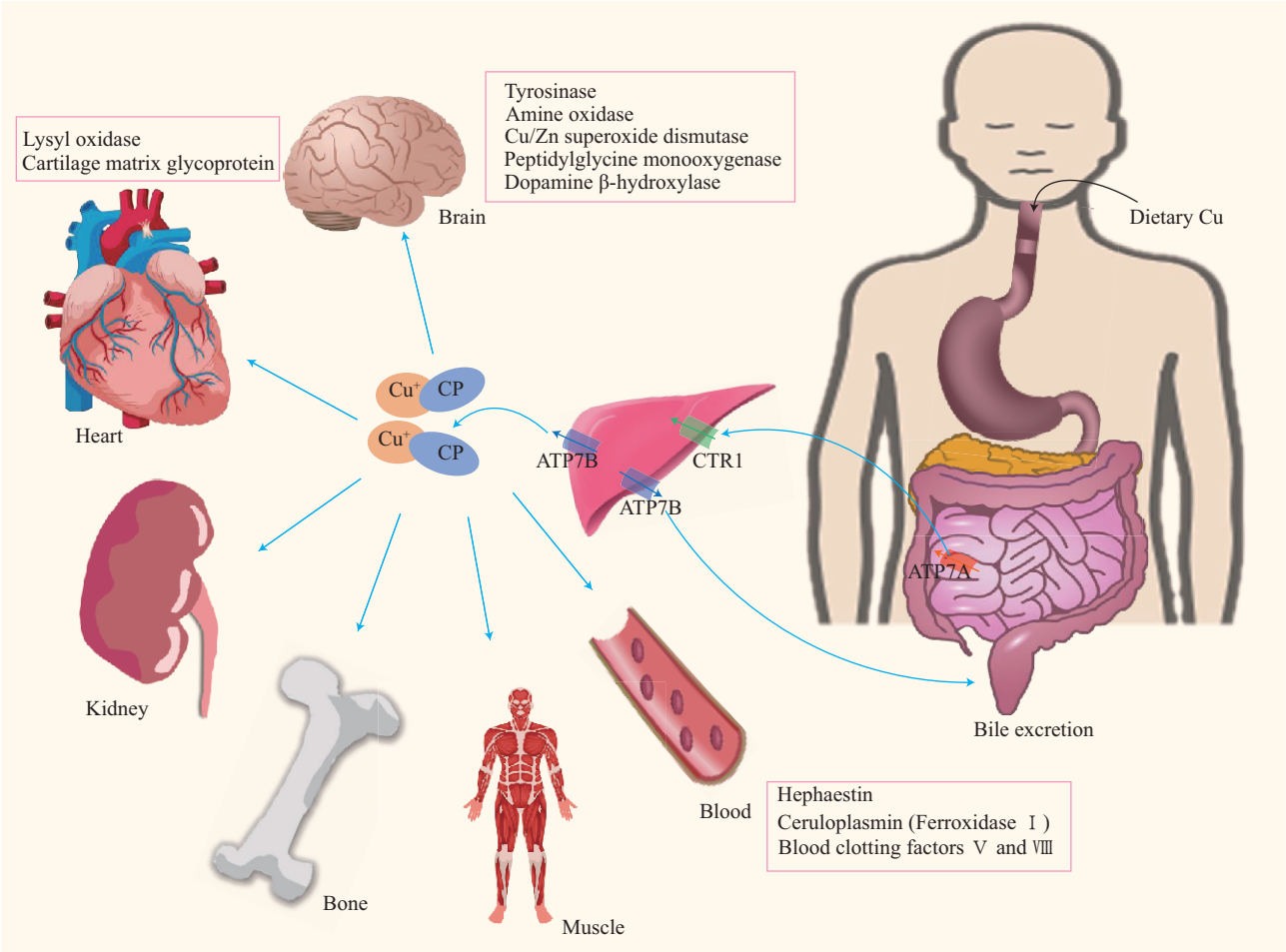

Fig. 1 The pathway of systemic copper (Cu) metabolism and key copper-containing enzymes in different organs
Cu is absorbed through the small intestinal epithelium, and transported into the portal circulation to reach the liver. Then, it is distributed to various organs and tissues in the body, including the brain, heart, kidneys, bone, muscle, and blood. Several of the listed Cu-containing enzymes are essential to the functions of different organs.

mitochondrial proteins, including cytochrome c oxidase (COX). COX comprises of 11 protein subunits, and requires 18 proteins for accurate assembly[41]. The catalytic center of COX comprises of COX1/2/3, which is encoded by mitochondrial DNA, and includes 3 copper ions. Two copper ions are situated in the CuA center, and one copper ion is located in the CuB center[42]. When COX17 binds copper, this transports the 2 copper ions to cytochrome c oxidase assembly protein 2 (SCO2). Then, SCO2 delivers the copper ions to SCO1 in the SCO2-SCO1-COX2 complex. Finally, the copper ions are transported to the CuA site at the catalytic COX2 center, in order to complete the subunit assembly. This process requires COA6[43]. COX17 links an additional copper ion to COX11, and this copper ion is transferred to the CuB site situated in the catalytic center of COX1, thereby concluding the COX1 assembly[44]. In addition, COX incorporates a heme group during metallization, followed by the integration of the remaining subunits, finalizing the COX assembly during oxidative phosphorylation[45]. Mitochondria generates and transfers a redox signal to regulate the transport of copper. Mutations in SCO1 and SCO2 may impact the integrity of this signaling pathway[46]. SCO1 deficiency induces the rapid degradation of CTR1, indicating the functional link between SCO1 and CTR1[47].

1.2.3 Copper Chaperone of Superoxide Dismutase (CCS) Copper binds to CCS, and delivers copper to SOD1. This promotes the catabolism of reactive oxygen species (ROS), reduces ROS accumulation, and protects cells from free radical damage. The deficiency of SOD1 would increase oxidative stress[48]. The expression of CCS is regulated by the negative feedback from copper. An increase in intracellular copper content would result in elevated CCS degradation[49]. Recent studies have suggested that copper can directly reach SOD1 from CTR1 *via* copper chaperones by forming the CTR1-CCS-SOD1 complex[50].

1.2.4 Antioxidant Protein 1 (ATOX1)/ATP7A/B ATOX1 binds copper, in order to deliver copper to ATP7B on the trans-Golgi network (ATP7A in other cells). This promotes the synthesis of copper proenzymes, including lysine oxidase, tyrosinase, and ceruloplasmin[51]. ATOX1 is a copper-dependent

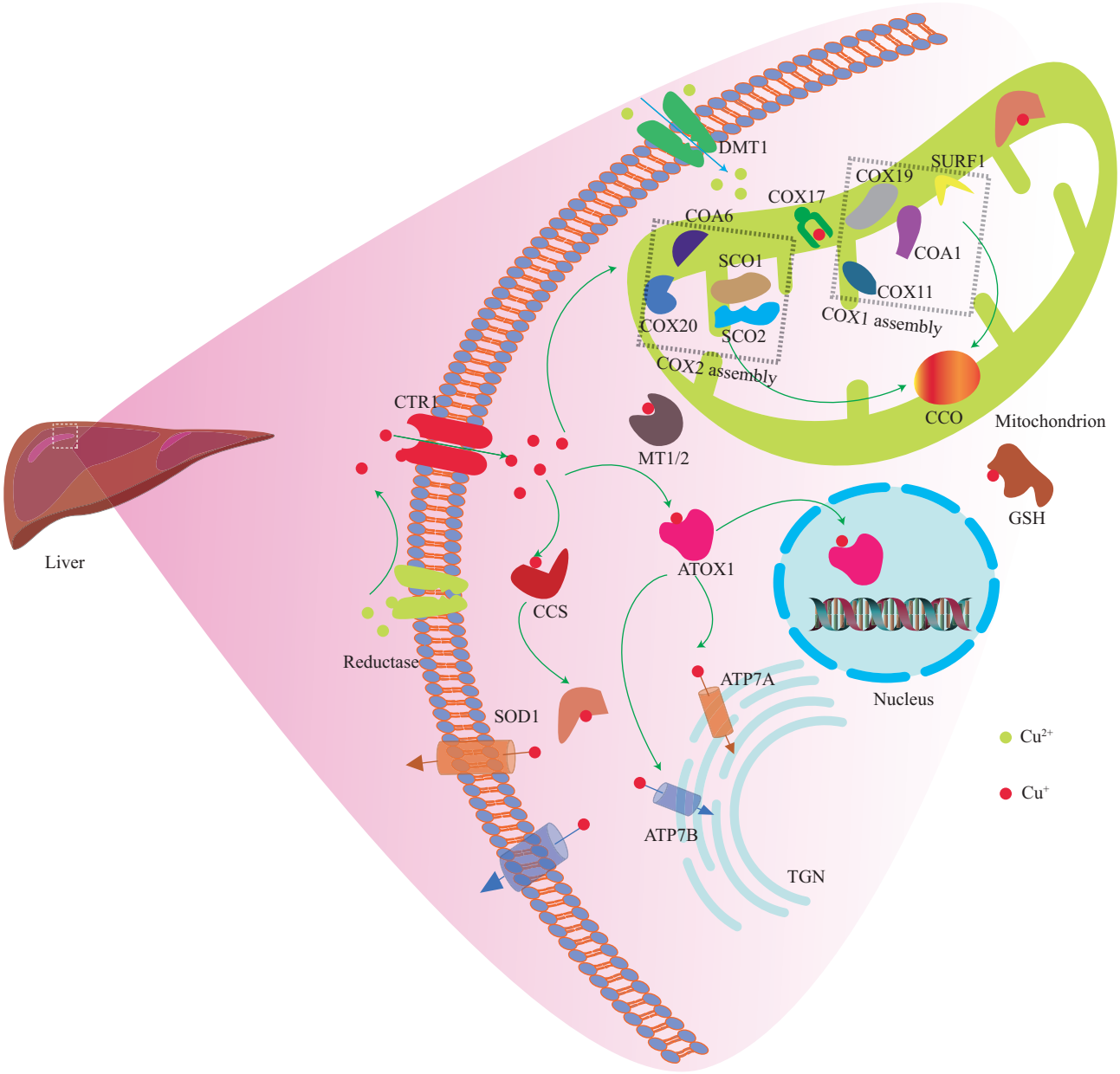

Fig. 2 The summary of the mechanism for regulating copper homeostasis in hepatocytes
Extracellular Cu^{2+} is reduced to Cu^{+} by the reductase (duodenal cytochrome b and six-transmembrane epithelial antigen of the prostate). Cu^{+} is transported into the cell *via* CTR1, and delivered to different copper chaperones to perform different functions. For example, CCS delivers Cu^{+} to SOD1, ATOX1 delivers Cu^{+} to the nucleus and Golgi, and COX17 delivers Cu^{+} to the mitochondria for CCO assembly. Meanwhile, intracellular metallothionein 1/2 and glutathione can chelate Cu^{+}. DMT1 can deliver Cu^{2+} into the cell. The delivery of Cu^{+} by COX17 is necessary for the assembly of both the COX1 and COX2 in the mitochondria. COX1 comprises of SURF1, COA1, COX11 and COX19, while COX2 comprises of SCO1, SCO2, COX20 and COA6. The mature holoenzyme complex comprises of these two parts, and incorporates a heme group during metallization, followed by the integration of the remaining subunits, finalizing the COX assembly during oxidative phosphorylation.

transcriptional regulator that contributes to cell multiplication. Mice that lack the *ATOX1* gene may encounter perinatal mortality as a result of abnormal copper homeostasis[52]. Intracellular ATP7A/B can regulate copper levels, and participate in copper transport between the cell membrane and various intracellular compartments in an ATP-dependent manner. ATOX1 interacts with the amino terminus of ATP7A/B to regulate its activity during copper transport by modulating the rate of ATP hydrolysis. In addition, the protein dynamically adjusts copper homeostasis, depending on the level of intracellular copper, with ATP7A re-localizing to the plasma membrane or ATP7B re-localizing to the vesicles, in order to facilitate the export of excess copper[34].

Copper binds to ATOX1 or other unidentified chaperone proteins to enter the nucleus for the regulation of several signal transduction pathways[53]. ATOX1 interacts with cysteine-rich protein 2 (CRIP2), and transfers copper to CRIP2, inducing

CRIP2 degradation, thereby raising the ROS levels, and activating autophagy[54]. In the nucleus, copper regulates the gene expression, and the subsequent protein synthesis by regulating transcription factors. Furthermore, copper regulates key transcription factors, including NF-κB. The treatment with different concentrations of copper chloride may induce the activation or inhibition of NF-κB[55]. Key transcription factors, including AP-1 and p53, are activated by excess copper[56].

2 COPPER HOMEOSTASIS IN THE HUMAN BODY

Both copper deficiency and copper overload can cause cellular damage. Therefore, the amount of copper in the body is maintained within a reasonable range, which is known as copper homeostasis[57]. Copper plays a crucial role in numerous metabolic processes for the orderly functioning of daily activities[58]. Evidence increasingly suggests that imbalances in copper homeostasis are associated with the development of several diseases, including Menkes disease[59], Wilson disease[60], neurodegenerative disorders, and cancer.

2.1 Copper Homeostasis in the Nervous System

The brain is the second largest copper-accumulating organ[61]. Copper homeostasis is equally important in the nervous system, and trace amounts of copper are necessary to perform normal brain development and function. Studies have elucidated the role of copper in the brain. Copper passes through the liver, and enters the brain. It serves as a cofactor in copper-dependent enzymes with important physiological functions, including the following: dopamine β-hydroxylase-like monooxygenase, which catalyzes norepinephrine synthesis[62]; cytochrome c mitochondrial oxidase, which has a mitochondrial role[63]; amine oxidases, which synthesize neurotransmitters[64]; tyrosinase oxidases, which form melanin[65]; sulfhydryl oxidase, which maintains the normal structure of hair[66]. Furthermore, copper is involved in other regulatory pathways in the nervous system, such as the synthesis of SOD1, which breaks down superoxide to protect cells from oxidative stress damage. Moreover, copper regulates the activity of amino acid and purine receptors[67]. In addition, copper can regulate synaptic transmission and related signaling by regulating ATP7A at synapses[68], as well as the calcium or zinc binding, and metallothionein expression[69]. It also regulates the function of brain-derived neurotrophic factors and nerve growth factors[70].

Copper distribution in the brain is uneven, with higher concentrations observed in both the locus coeruleus and substantia nigra[71]. The copper transportation and distribution within the brain remains unclear. It was hypothesized that copper crosses the blood-brain barrier (BBB) as free copper ions, and is released into the brain parenchyma and cerebrospinal fluid. The BBB appears as the primary route for copper entry into the brain parenchyma, and the BBB and blood-cerebrospinal fluid barrier likely maintains copper homeostasis in the brain. Furthermore, both BBB and blood-cerebrospinal fluid barrier cells express proteins involved in copper transport. Cells in the BBB express higher levels of copper transporter proteins, including CTR1, DMT1 and ATP7B than the brain parenchyma. Copper is transported more easily to the brain parenchyma *via* cerebral capillaries, as compared with transportation *via* the choroid plexus[72].

A recent study reported that the concentration of copper is higher in glial cells than in neurons[73]. Astrocytes, which are the most abundant glial cells found in the central nervous system, have been shown to have a significant impact on both health and diseases. Under typical circumstances, astrocytes participate in key physiological functions, including the regulation of developmental and functional synapse activity, and the BBB, the metabolic support for neurons, and the production of neurotrophic factors. Copper signaling pathways between neurons and astrocytes potentially play a significant role in brain signal processing[74]. It has been commonly considered that astrocytes absorb copper *via* CTR1. Additional studies have reported the involvement of DMT1 in copper uptake through astrocytes[75]. CTR1 and DMT1 facilitate the transportation of Cu^{+}. Astrocytes release small-molecule reductants *in vivo*, which reduce Cu^{2+} levels to aid the cellular uptake of copper[76]. Furthermore, the prion-related protein, which exhibits low-affinity binding to copper, may mediate copper uptake through astrocytes[77].

Significantly elevated copper levels have been identified in patients with Wilson disease[78]. Abnormal copper concentrations have been reported in patients with other neurodegenerative disorders, indicating the existence of specific copper signaling pathways in the brain. The complex copper signaling pathways within the brain include redox processes, which involve unstable copper ions, the release of neurotransmitters from synapses, and the cooperation between neuronal and glial cells. Regulating molecules, such as ATP7A/B[79], CTR1[80], and ATOX1[81], is a key function in copper homeostasis in the nervous system.

2.2 Copper Homeostatic Imbalance in Menkes Disease and Wilson Disease

During defective copper homeostasis in the body, copper deficiency or copper accumulation can lead to substantial damage, which has been linked to various diseases[82]. During embryogenesis and early development, adequate copper intake is important, especially in the central nervous system. Copper toxicity, due to excessive copper levels, particularly

affects the brain and liver[83].

2.2.1 Menkes Disease Menkes disease, which was first reported by Menkes in 1962[84], is a rare genetic disorder characterized by abnormal copper metabolism[85]. This disorder is inherited in an X-linked recessive manner, and is the result of mutated ATP7A. *ATP7A* gene mutations result in the impaired intestinal function of ATP7A, leading to the reduced entry of copper into the bloodstream, and accumulation of copper in intestinal cells[59]. If copper cannot be transported to the different organs of the body, including the liver and brain, this would ultimately lead to severe systemic copper deficiency, and impaired synthesis of several important enzymes[86]. For instance, the impairment of dopamine β-hydroxylase-like monooxygenase function may result in defective synaptic function and axonal growth[87]. The pathology of the tetralogy of Fallot is characterized by inborn vascular anomalies in newborns[88]. Patients with Menkes disease often present with symptoms, such as bone loss, skin laxity, aneurysms, and spontaneous fractures[89].

A study of *ATP7A* gene mutations suggested that genetic screening is a reliable diagnostic method for Menkes disease[90]. The treatment of Menkes disease depends on the copper absorbed through the gut, and the amount of circulating copper that reaches the brain[91]. For example, the prompt subcutaneous injection of Cu-histidine complexes to overcome intestinal absorption barriers can enhance the treatment outcomes[92].

2.2.2 Wilson Disease Wilson disease is a genetic mutation-causing disease, which features the pathological accumulation of copper[60]. The pathogenesis of Wilson disease is primarily attributed to mutations in the *ATP7B* gene, which leads to the inactivation of the transmembrane copper-transporting ATPase, the obstruction of copper excretion from the biliary tract, and the eventual disruption of copper homeostasis[93]. Furthermore, copper overload in hepatocytes leads to cirrhosis and liver fibrosis[94]. The copper released from liver cells gradually accumulates in other organs, and leads to extrahepatic toxicity[95].

Copper concentrations in patients with Wilson disease may be 10–15 folds higher than those in healthy individuals[96], suggesting the strong association between copper levels in the brain and neuropathological severity. The toxic effects of copper are initially buffered by astrocytes, accompanied by its proliferation. The synthesis of metallothionein is subsequently upregulated to increase its copper storage ability. Eventually, high copper levels would lead to astrocyte impairment, BBB dysfunction, and diverse brain tissue pathologies[97]. Morphological and functional abnormalities of the retina in Wilson disease are correlated to the severity of brain pathology and neurological impairment[98].

The diagnosis of Wilson disease is based on clinical symptoms, the measurement of copper metabolism, and the analysis of *ATP7B* genes. Copper overload can be reversed *via* chelation therapies and oral zinc. D-penicillamine and trientine can increase urinary copper excretion, while oral zinc can reduce copper absorption in the digestive tract[99]. Accurate and early pharmacological treatment can lead to improved liver function and transaminases within 2–6 months, and neurological improvements can be observed in 50%–60% of patients within 1–3 years[100].

2.3 Copper Homeostasis and Neurodegenerative Diseases

Numerous studies have reported that changes in copper homeostasis occur during progressive neurodegenerative diseases[73]. Both the increase and decrease in copper levels may play a distinct role in neurodegenerative diseases.

2.3.1 Alzheimer's Disease (AD) and Copper Homeostasis AD is a prevalent neurodegenerative disorder that may stem from diverse factors, such as age, environment, and genetics. The increase in human life expectancy in the coming years would likely result in a growth in the number of patients with AD[101]. According to the conventional "amyloid cascade hypothesis", AD pathology is primarily mediated *via* the accumulation of amyloid-β (Aβ) peptide and tau proteins. Subsequently, this defective processing would result in the formation of amyloid plaques and downstream neurofibrillary tangles in the grey matter, accompanied by cellular oxidative stress, vascular injury, neuroinflammation, and neurodegenerative lesions[102, 103].

Copper homeostasis plays a pivotal role in the pathogenesis of AD. Copper potentially interacts with several key pathological factors, including Aβ and tau. Aβ binds to copper ions with high affinity[104]. Copper ions can induce Aβ precipitation *in vitro*. However, isolated copper ions stimulate Aβ degradation, and impair the production of hydroxyl radicals and oxidative damage, ultimately reducing cell death[105]. Excessive copper can impair the brain's capacity to eliminate Aβ. Furthermore, the Aβ-copper complex inhibits the expression of lipoprotein receptor-related protein-1, thereby diminishing the removal of neurotoxic Aβ[106]. Copper has been identified to be significantly enriched in age spots of patients with AD, indicating that this may play a role in triggering plaque formation in the brain[107]. Furthermore, copper enhances the phosphorylation of tau proteins and the neurotoxicity of tau protein aggregates. The tau proteins that are bound to copper promote brain damage by inducing redox activity[108]. Chronic exposure to systemic copper leads to the dysregulation of tau-related kinase CDK5 and synaptic protein complexin1/2[109]. Studies have reported conflicting findings on brain copper levels in AD. However, elevated levels of labile copper may

contribute to oxidative tissue damage in the brain of patients with AD[110].

In cases of neuroinflammation, copper exacerbates the impact of Aβ on microglial activation, followed by neurotoxicity. Copper-Aβ complexes activate microglia, and facilitate the release of TNF-α and NO in an NF-κB-dependent manner[111]. Furthermore, copper-Aβ complexes participate in the TNF-α signaling pathway and concurrent caspase-3 activation that results from oxidative stress, leading to neuroinflammation[112]. It has been proposed that copper modifies the pro-inflammatory and anti-inflammatory phenotypes of microglia by regulating the NO and S-nitrosothiol signaling pathways[113]. In addition, neuronal death can drive inflammation in AD. Different types of neuronal death affect inflammation *via* different mechanisms, such as neuronal apoptosis, leading to the disruption of normal microglial homeostasis[114–118]. Necroptosis, pyroptosis, and ferroptosis represent soluble cell death characterized by the release of DAMPs, including HMGB1, heat shock proteins, and nucleic acid[119–122]. This causes sustained neuroinflammatory damage, and triggers aberrant microglial activation[123]. Interventions against neuronal death may improve the neuroinflammatory environment in AD. Furthermore, the timing and mechanism of neuronal death may provide insights into the treatment of neurodegenerative diseases.

Astrocytes store significant amounts of copper. *In vitro* evidence has revealed that astrocytes with impaired function accumulate copper *via* a CTR1-dependent mechanism or DMT1, and the ZIP family of proteins[124]. Free copper may induce degenerative neuronal lesions[125]. The enrichment of copper chaperone proteins in astrocytes may impair copper transport from astrocytes to neurons[126]. *In vitro* experiments have indicated that Aβ plaques and protofibrils act as endogenous stimuli, leading to astrocyte activation or reactive astrocyte proliferation[127]. Astrocytes can impede the microglia-mediated clearance of Aβ plaques by secreting glycosaminoglycan-sensitive molecules, indirectly promoting Aβ accumulation in the AD brain[128]. In addition, the administration of Cu^{II}(atsm) can substantially decrease the secretion of NO, MCP-1, and IL-6 in astrocytes, which may be linked to the amplification of cellular copper and metallothionein-1 in astrocytes[129].

The antioxidant glutathione is critical in AD. This is frequently used to demonstrate mild cognitive impairment and AD pathology[130]. It has been considered that elevating glutathione levels may impede or decelerate the progression of AD. High levels of copper have been considered to decrease the production of glutathione, leading to an excess of oxidative free radicals, and consequent oxidative stress damage, which may contribute to the pathogenesis of AD[131]. Another significant protein involved in AD is SOD1, in which the Cu^{2+} binding site is similar to that of Aβ. As a result, Aβ may impair the structural integrity of SOD1, leading to defective cellular metal scavenging[104].

AD has been managed with some small molecules, including donepezil, galantamine, rivastigmine, and memantine. However, no permanent cure exists for AD. These drugs are potential antioxidants, which can be used to decrease Aβ aggregation, and improve neurological symptoms[132]. An experimental drug, clioquinol, was identified to decrease Aβ deposition after 3 months of oral administration in animal models[133]. In one clinical trial, 36 patients with moderate AD experienced relief in cognitive decline with chloroquine[134]. A number of clinical trials associated with neurodegenerative diseases are undergoing (table 1).

2.3.2 Huntington's Disease (HD) and Copper Homeostasis HD is an autosomal dominant disorder of the nervous system[135]. Abnormal repeats of the N-terminal polyglutamine sequence of Huntingtin protein is the molecular mechanism underlying HD pathogenesis. The mutated Huntingtin proteins aggregate, and result in oxidative stress and neurodegenerative symptoms[136].

In both people and animals with HD, the striatum has been shown to have increased levels of copper ions[137]. Some studies have indicated that copper buildup enhances the deleterious functions of mutant proteins[138]. In addition, copper binds to histidine residues located at the N-terminal end of Huntingtin proteins[139]. Remarkably, merely copper exhibits a binding affinity towards Huntingtin proteins that comprise of 17–68 glutamine residues. The use of copper chelators hinders the formation of clusters of mutant Huntingtin proteins, while amplified copper consumption facilitates the creation of clusters[140]. Copper might also contribute to advanced HD by obstructing enzymes associated with mitochondrial respiration[141]. A decrease in lactate removal occurs in the striatum of patients with HD[142], and research has shown that the use of lactate dehydrogenase inhibitors can induce neurodegeneration in mice[143].

Copper chelators, such as clioquinol, tetra-thiomolybdate, and bathocuproine disulfonate, can alleviate HD[144]. The use of these chelators in mouse models of HD significantly attenuated the pathological and behavioral abnormalities, and enhanced the survival rates in Drosophila models of HD[139].

2.3.3 Amyotrophic Lateral Sclerosis (ALS) and Copper Homeostasis ALS leads to selective motor neuron degeneration and subsequent death[145]. Its primary clinical feature is progressive muscle atrophy and weakness, leading to respiratory failure and death in patients[146]. The cause of ALS remains uncertain. A genetic etiology has been suggested in 20% of cases,

Table 1 Clinical trials in copper-related neurodegenerative diseases (data obtained from ClinicalTrials.gov)

Interventions	Condition	Study phases	Results	Location
GE180 PET Scan	AD	Ⅱ	GE180 was used to analyze the regional and global inflammation in the brain of patients with AD and PD, and greater whole brain GE180 was found to be correlated to poorer cognitive function, including the frontal/cingulate/parietal/temporal lobe.	Nevada, USA
Gastro-retentive zinc cysteine tablet	AD	Ⅱ	The orally administered active comparator material was associated with better tolerability, when compared to oral zinc acetate, and it induced a reduction in serum non-ceruloplasmin bound copper levels, and an elevation in serum zinc levels	Florida, USA
8 mg/day of copper	AD	Ⅱ	Changes were found in cognitive function, beta-amyloid in the CSF, and volumetric in the brain.	University Hospital, Saarland
2 mg/day of copper	ALS	Ⅱ	No posted	Arizona, United States
$Cu^{Ⅱ}$(atsm)	ALS	Ⅱ	No posted	New South Wales/Victoria, Australia
$Cu^{Ⅱ}$(atsm)	MS	Ⅰ	No posted	
$Cu^{Ⅱ}$(atsm)	PD	Ⅰ	The drug dose was 12 mg/day, which was well-tolerated in the ALS study.	
Coconut oil-epigallocatechin gallate	MS	Ⅱ	No posted	Valencia, Spain
Multimodal exercise program	PD	No	No posted	Chang Gung Memorial Hospital
Observational study: copper exposure	PD		No posted	Isernia/Napoli, Italy
Managing fatigue: The Individual program (MFIP)	PD	No	No posted	Nova Scotia, Canada
Not researched	HD			

AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; MS: multiple sclerosis; PD: Parkinson's disease; HD: Huntington's disease

while environmental factors, neurotoxin accumulation, oxidative stress damage, and inadequate growth factors may account for 80% of patients with ALS[147].

The mutant SOD1 protein may play a role in familial ALS pathogenesis. During the progression of ALS, CCS erroneously binds to mutant SOD1, thereby decreasing the copper delivery to mitochondria, and leading to the buildup of abnormal SOD1[148]. In turn, this generates motor neuronal toxicity[149]. The overexpression of CCS in the *SOD1*^{G93A} mutant mouse model expedites neurological deficits, and shortens the survival time of mice, while the administration of copper ionophore ameliorates the symptoms[150]. The increased expression of CCS alters cytochrome c oxidase activity in SOD1 mutation[151]. The additional toxicity induced by the CCS overexpression increases the delivery of copper to SOD1, but decreases other deliveries, thereby affecting the normal function of other enzymes[152]. Thus, the dysregulation of copper homeostasis and impairment of copper-dependent enzyme function may contribute to the acquired toxicity in patients with mutant SOD1, and the development of ALS. Elevated copper concentrations in skeletal muscle and the spinal cord were identified to accompany disease progression during the pre-symptomatic phase in *SOD1*^{G93A} mutant mice[153]. Excess copper was detected in the cerebrospinal fluid in people and animals with ALS[154]. The expression of human copper transporter 1 augmented the copper concentrations within the spinal cord, thereby reinstating SOD1 and ceruloplasmin activity[155]. The more severe the copper deficiency in mutant SOD1, the more severe the clinical symptoms of ALS[156].

Several copper chelators, including D-penicillamine[157] and tetrathiomolybdate[158], have demonstrated a palliative effect on ALS. In addition, diacetyl-bis (4-methylthiosemicarbazonato) copper (Ⅱ) [$Cu^{Ⅱ}$(atsm)], which is a copper ionophore, has been identified as a significant drug for treating ALS[159]. $Cu^{Ⅱ}$(atsm) has been shown to enhance motor function and improve livability in *SOD1*^{G93A} and *SOD1*^{G37R} mutant mouse models[160]. In the *SOD1*^{G93A} mutant mouse model, the treatment with tetrathiomolybdate has been shown to extend survival, alleviate muscle atrophy, and reduce motor neuron loss, while inhibiting the activity of SOD1 mutant proteins, and diminishing mutant protein aggregation[161].

2.3.4 Parkinson's Disease and Copper Homeostais The primary clinical manifestations of PD include resting tremors, muscle tone modifica-tions, brady-kinesia, and postural instability[162]. The disease

pathology is characterized by the reduction of dopaminergic neurons, and the formation of protein aggregates that comprise of α-synuclein protofibrils[163]. Several genetic mutations of α-synuclein may exist in hereditary PD[164]. PD occurs in both inherited and sporadic forms. Various factors, such as aging and the environment, may affect PD development[165]. It has been widely considered that the combination of genetic susceptibility and environmental factors triggers PD[166]. Furthermore, oxidative stress injury and mitochondrial dysfunction were considered to promote PD progression[167].

Autopsies have shown that the brains of patients with PD carry higher levels of oxidative damage to proteins, DNA, and lipids than healthy brains[168]. In addition, glutathione levels in the substantia nigra of patients with PD decreased by 40%–90%[169]. Alpha-synuclein oligomers form a toxic amyloid conformation[170]. Initial *in vitro* experiments have revealed that millimolar concentrations of copper facilitate the development of partially-folded amyloid heterodimers, rendering them increasingly vulnerable to aggregation[171]. The N-terminal domain of α-synuclein contains a copper-binding site[172]. This process is enhanced by reducing the electrostatic repulsion of negative charges[173]. N-terminal acetylated α-synuclein was recently discovered in the brain[174]. This modification promotes the protein's helical folding, and decreases protein aggregation[175]. Although this modification did not affect the ability of α-synuclein to bind Cu^{+}, this interfered with its binding to Cu^{2+}[176]. Copper ions act as a double-edged sword, and reduced copper levels may be involved in PD progression[177]. Copper levels are lower in brain regions with the most damage in patients with PD, including the substantia nigra and locus coeruleus, with a reduction of 35%–50%, when compared to healthy brains[178].

The mechanism of copper metabolism in PD suggests that PD can be alleviated *via* copper reduction or supplementation. A compound 8-hydroxyquinoline-2-carboxaldehyde isonicotinoyl hydrazone can cross the BBB, and competitively bind to Cu^{+} or Cu^{2+}, effectively inhibiting the protein aggregation *in vitro*[179]. Clinical trials on Cu^{II}(atsm) have suggested its accumulation in the striatum of patients with PD during disease progression[180]. Furthermore, it was found that Cu^{II}(atsm) can be used to restore copper-deficient SOD1 function, and rescue neuronal loss. In addition, the testing results for the genetic mouse model of PD revealed that Cu^{II}(atsm) rescued the dopaminergic cell loss, and improved motor dysfunction[151].

3 CUPROPTOSIS IN THE HUMAN BODY

3.1 Definition of Cuproptosis

A 2022 study investigated the mechanisms of copper homeostasis, and reported that copper binds to the lipoylated proteins of the TCA cycle[14], inducing lipoylated protein aggregation, and resulting in proteotoxic stress and cell death. For the first time, cuproptosis was proposed, which is regulated by copper during mitochondrial respiration. This form of death contrasts with all other recognized forms of cell death[181–184].

Copper ionophore elesclomol binds copper to facilitate its transportation into cells[185]. This can be used to investigate copper toxicity. Further studies have demonstrated that cell death induced by copper ionophore is merely contingent on high levels of copper[186]. Elesclomol triggers ROS-dependent apoptosis[187], although caspase 3, an apoptotic marker, remains inactive during elesclomol-induced cell death. The involvement of multiple death inhibitors, or the removal of BAX and BAK1 did not alter the probability of death, suggesting that this may be a new type of cell death. Cells that are dependent on mitochondrial respiration are approximately 1000 folds more responsive to copper ionophore than glycolysis-dependent cells[188]. This implies that copper ionophore-induced cell death is potentially linked to mitochondrial respiration. A genome-wide CRISPR/Cas9 loss-of-function screening revealed 7 genes that successfully rescued copper ionophore-induced cell death, including *FDX1*, which is the direct target of elesclomol[189]. *FDX1* encodes a reductase that reduces Cu^{2+} to Cu^{+}. *LIPT1*, *LIAS*, and *DLD* encode the lipoic acid pathway, while *DLAT*, *PDHA1*, and *PDHB* are responsible for protein lipoylation[190]. The knockdown of *FDX1* and *LIAS* leads to the resistance against copper-induced cell death. The concurrent screening of databases and immunohistochemical studies revealed a high degree of correlation between FDX1 and proteins in the lipoic acid pathway, suggesting that FDX1 might serve as an upstream regulator of proteolipid acylation. Toxic gain of function may occur due to copper binding to lipoylated TCA proteins, which is potentially induced by the abnormal oligomerization of lipoylated proteins. Mass spectrometry analysis has demonstrated that copper toxicity can lead to the loss of FDX1-dependent iron-sulfur cluster proteins and proteotoxic stress. However, this remains to be explored. Finally, the conclusions of this study were validated using a cellular model that overexpressed the SLC31A1 protein, and an aged *ATP7B*$^{-/-}$ animal model[191] (fig. 3).

The determination of the regulatory mechanisms of diseases induced by the imbalance in copper homeostasis remains challenging. Oxidative stress damage has been investigated in a significant number of studies[192]. Copper ionophore NSC319726[193] activates copper, promoting the production of ROS[194]. The activation of autophagy was observed to protect

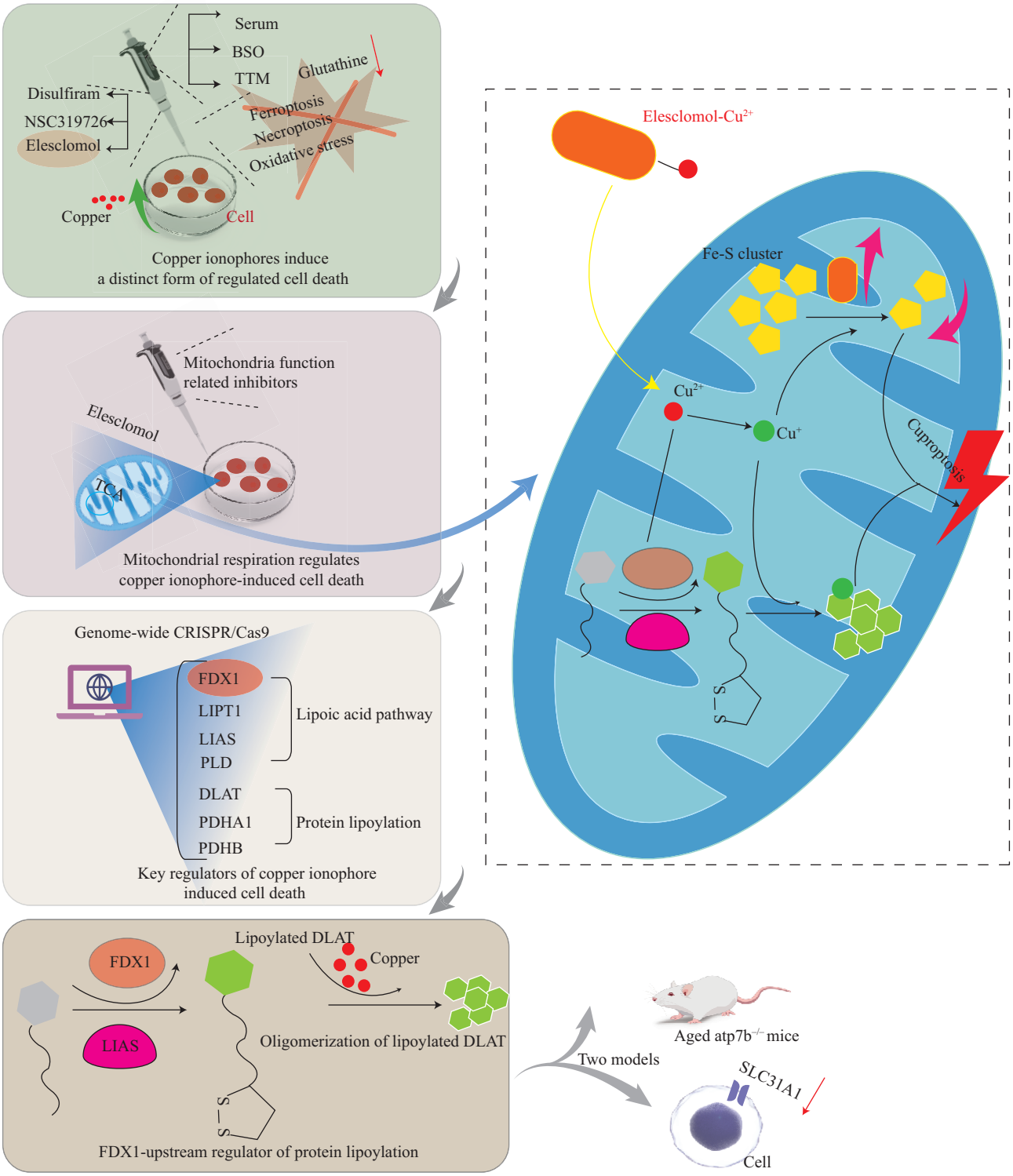

Fig. 3 The initial discovery process and mechanism of cuproptosis

The experiments suggest that the cell death induced by elesclomol might be a novel cell death type. Cells cultured with serum exhibited higher sensitivity towards elesclomol. After the depletion of glutathione *via* BSO, the cells exhibited heightened sensitivity to elesclomol. However, the chelation of copper ions through the TTM rescued the elesclomol-induced cell death. After the 2-h pulse treatment with elesclomol, DSF and NSC319726, the intracellular copper levels increased by 5–10 times. Cell death occurred for more than 24 h following treatment. The use of several cell death inhibitors did not affect the onset of copper-induced death. The treatment with mitochondrial antioxidants, fatty acids, and inhibitors of mitochondrial function had a significant impact on cell viability. However, mitochondrial uncoupler FCCP did not affect the cell survival. A genome-wide CRISPR/Cas9 loss-of-function screening identified 7 genes that rescued the copper ionophore-induced cell death. Mitochondrial respiration regulated this novel copper ionophore death, and a key regulator, FDX1, was identified. Copper binds to the lipoylated components of the TCA cycle, inducing lipoylated protein aggregation, and resulting in the loss of iron-sulfur cluster proteins, proteotoxic stress, and ultimately, cell death. BSO: buthionine sulfoximine; DSF: disulfiram; NSC319726: copper ionophore; TTM: tetrathiomolybdate

hepatocytes from copper-induced death in liver tissues of individuals with Wilson disease, and in ATP7B-deficient animals[195]. In another study, copper stimulated the development of tumors by activating the PI3K-AKT signaling pathway. Reducing CTR1 or inhibiting the CTR1-Cu axis with copper chelators can reduce tumor development and AKT signaling. Furthermore, CTR1 is negatively regulated by Nedd4l. These results established a link between Nedd4l-CTR1-Cu and PDK1-AKT oncogenic signaling[196]. MTF1 is a classic metal-binding transcription factor that plays a key role in cuproptosis[197]. Furthermore, *p53*, which is a widely expressed oncogene, regulates iron-sulfur cluster proteins and glutathione synthesis[198]. Cuproptosis-induced inflammation is also under investigation, with HMGB1 as a significant immune mediator[199]. In addition, cuproptosis may be associated with autophagy[200].

Cuproptosis is considered as a potential therapeutic target in cancer. Numerous recently published studies have investigated cuproptosis using cancer databases and bioinformatics approaches. Different models have been used to study the risk of cancer, and investigate tumor immunity, treatment, and prognosis. For instance, in glioblastoma, epigenetic regulatory proteins may regulate cuproptosis by altering the expression of PD-L1 and FDX1[201]. In uveal melanoma, copper is translocated to the mitochondria, generating large amounts of ROS, and inducing cancer cell migration[202]. The *LIPT1*, *PDHA1*, and *SLC31A1* linked to cuproptosis were upregulated in melanoma[203]. A total of 10 cuproptosis-associated lncRNAs exhibited higher diagnostic efficiency in clear cell renal cell carcinoma[204]. The FDX1 expression was significantly downregulated in hepatocellular carcinoma (HCC), while its elevated expression was linked to extended survival[205]. LIPT1 may stimulate the growth, infiltration, and movement of HCC cells[206]. In low-grade gliomas, an accurate prognostic model based on 5 genes related to cuproptosis[207] was built, and it was found that the expression of ATP7B decreased, while the expression of SLC31A1, FDX1, DLAT, and LIAS increased[208].

The study of cuproptosis has been increasingly utilized for therapeutic purposes. A ROS-sensitive polymer was developed to encapsulate elesclomol and copper in nanoparticles, which in turn, are activated by excessive intracellular ROS upon entry into cancer cells. The elesclomol and copper complexes synergistically act against cancer cells and induce cuproptosis[209]. The glucose oxidase-engineered nonporous copper (I) 1,2,4-triazole coordination polymer nanoplatform, which is also known as GOx@[Cu(tz)], was engineered in bladder cancer. This can make cancer cells more susceptive to cuproptosis when glucose and glutathione are depleted[210]. A hollow amorphous bimetal organic framework can be developed to leverage the synergistic effects of cuproptosis and ferroptosis against cancer[211].

3.2 Link Between Cuproptosis and Ferroptosis

Copper and iron have similar structures, and are both essential for the functioning of an organism[212, 213]. The imbalance in copper and iron levels typically leads to the generation of detrimental oxidative free radicals. Similar to copper homeostasis, the body tightly regulates iron homeostasis[214]. Defective iron homeostasis can lead to ferroptosis, which is a regulatory cell death identified in 2012[215]. The main mechanism involves the catalysis of highly unsaturated fatty acids on the cell membrane in the presence of divalent iron or lipoxygenase, resulting in lipid peroxidation and cell death[216]. An overlap between copper and iron homeostasis has been observed, and the mechanisms that regulate iron and copper have been identified. Copper has a positive impact on iron homeostasis, while iron hinders copper metabolism. For instance, following the depletion of iron stores in the body, copper is redistributed to tissues crucial for maintaining iron homeostasis, thereby facilitating iron absorption with the help of the divalent metal ion transporter DMT1[217]. In addition, gut-based copper may enhance iron transportation, with hepatic copper promoting the synthesis of ceruloplasmin, thereby enabling the oxidation of iron after its release[218]. During iron deficiency, hypoxia-inducible transcription factor (HIF) transactivates numerous intestinal genes that are linked to iron absorption[219]. Copper increases the DNA-binding activity of HIF, suggesting a possible link between the HIF signaling pathway, and both iron and copper homeostasis[220]. Studies have revealed that excess iron can affect normal copper metabolism[221]. Thus, it is necessary to emphasize the influence of iron consumption on copper homeostasis.

Studies have revealed that the chelation of copper ions by cuprizone results in the rapid release of iron ions from the storage protein, ferritin, which leads to iron-induced lipid peroxidation and ferroptosis, and results in the loss of oligodendrocytes[222]. A recent study revealed that copper homeostatic regulator COMMD10 regulates the onset of ferroptosis[223]. The disulfiram-copper complex can trigger ferroptosis in cancer. This significantly activates p62 phosphorylation, and promotes the competitive binding of Keap1, thereby prolonging the half-life of NRF2, and inducing the compensatory elevation of NRF2[224]. Copper stimulates ferroptosis by inducing the autophagic degradation of GPX4. TAX1BP1 acts as an autophagy receptor for GPX4 degradation or copper-induced ferroptosis[225]. Various studies have identified the correlation between cuproptosis and ferroptosis in different tumors, such as lung adenocarcinoma, in which 3 Cu-Fe clusters associated with cuproptosis and ferroptosis were identified[226]. In colorectal cancer, patients with

low expression of genes linked to cuproptosis and ferroptosis had higher survival rates[227]. In HCC, 7 key genes that linked cuproptosis and ferroptosis were identified as biomarkers of poorer prognosis[228].

3.3 Cuproptosis and Neurodegenerative Diseases

A number of studies have revealed the correlation between changes in copper homeostasis and the progression of various neurodegenerative disorders[229]. In AD, the key pathology protein Aβ has high affinity for binding to copper ions, and excess copper interferes with the removal of Aβ. High levels of copper ions have been detected in the striatum of HD patients. In ALS, CCS erroneously binds to mutant SOD1 proteins, resulting in incorrect copper delivery and anomalous SOD1 accumulation, and subsequently triggering the toxicity of motor neurons. For patients with PD, copper may accelerate the disease progression through multiple mechanisms mediated *via* increased or reduced copper levels. The precise mechanism of copper in advanced neurodegenerative disorders requires further investigation[230]. Recent studies have established a connection between the dysregulation of copper homeostasis in patients with Menkes disease, and the *UCHL1*/*PARK5* pathogenic pathway in PD. UCHL1/PARK5 is located downstream or parallel to ATP7A, indicating that the inhibition of UCHL1/PARK5 protects ATP7A mutants against the dysregulation of copper homeostasis[231]. A novel ATP7A substitution variant, p.Met1311Val, was identified in individuals with ALS, which increased copper accumulation in fibroblasts, decreased survival, and induced motor defects in Drosophila motor neurons[232]. A study that investigated Menkes disease and Wilson disease reported a novel method of regulating copper homeostasis. The findings suggested that ATP7 proteins and the conserved oligomeric Golgi complex together stabilize the copper levels, thereby contributing to the maintenance of mitochondrial function and synaptic integrity[233].

Since copper excess induces cuproptosis, which mainly occurs in mitochondria, and triggers oxidative stress injury, it is plausible that a potential pathogenic mechanism underlying various neurological disorders is mediated *via* cuproptosis. Notably, studies that used clioquinol have reported positive therapeutic outcomes for PD and AD, including decreased activation of microglia in the spinal cord with encephalomyelitis, leading to enhanced clinical symptoms[234]. A study that investigated the progression of neurological diseases based on the association between cuproptosis and ferroptosis reported that copper triggers ferroptosis. This induces oligodendrocyte loss in multiple sclerosis, and leads to cell death in other neurological diseases[222]. Cuproptosis-related genes have been reported in AD, and significant immune heterogeneity in patients with AD across various subgroups of cuproptosis has also been reported. In addition, *MYT1L*, *PDE4D*, *SNAP91*, *NPTN*, and *KCNC2* have been identified as unique genes with predictive capabilities in the AD analysis[235] (table 2).

4 DISCUSSION

Copper-based nanoparticles have been widely used in production life due to their outstanding properties[236]. In the biomedical field, CuO nanoparticles can be used as biosensors for the detection of disease markers[237]. Recently, CuO nanoparticles have been developed as antiviral surface coatings to inhibit viral transmission[238]. However, excess copper has adverse effects. A study revealed that prolonged exposure to copper may cause cognitive decline, and the occurrence of AD[239]. Similarly, the widespread use of high doses of copper in agriculture and livestock can lead to serious environmental pollution, ultimately endangering human health[240]. Therefore, it is crucial to establish rational laws for copper emission, and reduce copper use in organic agriculture.

Cuproptosis depends on the imbalance in copper homeostasis, suggesting the need for further investigation into the underlying mechanisms, such as additional regulators associated with membrane transport, and the distribution of varying levels of copper. A possible hypothesis is that the structure of copper transporters may be altered, or oligomeric modifications may be induced to regulate the transport pathway upon the binding of copper[241]. COMMD1 is the only identified membrane transport regulator that has copper binding capacity, and is a direct regulator of cellular copper homeostasis[242]. It has been reported that mice with liver-specific defects that involved COMMD1, COMMD6, and COMMD9 induce similar copper accumulation in the liver. COMMD6 and COMMD9 may play a role similar to COMMD1[243], suggesting the presence of other unknown modulators that specifically regulate copper transport. Therefore, the following questions remain to be addressed: (1) How do transport mechanisms sense copper levels to regulate the distribution of copper transporters? (2) Is there a copper-specific regulatory mechanism responsible for regulating the membrane transport of copper? (3) What is the intracellular copper homeostatic mechanism between organelles? (4) What are the differences in copper transport between neuronal and non-neuronal cells? A suitable neuronal model is required to revisit the mechanism of copper transport. Several key regulators involved in cuproptosis have revealed a connection between ferroptosis and autophagy. For example, in glioblastoma, *FDX1* with other related genes is linked to the expression of autophagy markers[200]. Copper can bind with related proteins to trigger autophagy[54]. In ATP7B mutants, the

Table 2 Some signaling pathways of copper homeostasis and cuproptosis in neuropathological conditions and tumors

Regulators of signaling pathways or molecules targeted by Cu	Function	Disease	Possible signaling pathway	Other findings	References
EREG	EREG can influence immunity and cuproptosis	Glioblastoma	EREG affects the expression of PDL1/related to cuproptosis by influencing the expression of FDX1.		[201]
FDX1	Associated with immune infiltration	Glioma	Cuproptosis genes related to FDX1 were positively correlated to the expression of autophagy marker genes Atg5, Atg12, and BECN-1.	LIPT2 and NNAT, which are involved in lipoylation, may be the unidentified marker genes for cuproptosis.	[200]
MAP1LC3A	Autophagy regulator, MAP1LC3A known as LC3	Wilson disease	Copper increased the expression of MAP1LC3A.	In ATP7B-knockout cells, mTOR is less active, and is dissociated from lysosomes. The mTOR substrate transcription factor EB translocates into the nucleus, and autophagy-related genes are activated to protect cells from copper-induced death.	[195]
ATP7	ATP7A and ATP7B proteins localize to the Golgi, and regulate copper homeostasis.	Neurodegeneration	The integrity of Golgi-dependent copper homeostasis mechanisms, which require the ATP7 and COG complex, is necessary to maintain mitochondria functional integrity.		[233]
ATP7A	The mutation of ATP7A can cause Menkes disease from systemic copper depletion.	Menkes disease	Copper dyshomeostasis, due to defects in ATP7A, increases the expression of UCHL1, which in turn, is required for the pathomechanism of copper dyshomeostasis.	A connection between copper dyshomeostasis and the UCHL1/PARK5 pathway of Parkinson disease	[231]
CTR1	CTR1 is a copper transporter in the cell membrane.	Cancer	Copper promotes tumorigenesis by activating the PDK1-AKT oncogenic pathway in a CTR1-dependent manner.	Nedd4l negatively regulated CTR1 through ubiquitination and subsequent degradation/ Nedd4l-CTR1-copper-PDK1-AKT.	[196]
Ferritin	Ferritin is an iron storage protein.	Demyelination injury	Cuprizone chelates copper and rapidly mobilizes iron from ferritin, which triggers iron-mediated lipid peroxidation and oligodendrocyte loss through ferroptosis.	CZ induces demyelination *via* the ferroptosis-mediated rapid loss of oligodendrocytes.	[222]
MTFI	Classical metal binding transcription factors are closely related to copper homeostasis and cuproptosis.	Copper loading or deficient	Copper loading induces the transcriptional activation of MT through the MTF1 and MRE-dependent pathways, and promotes the nuclear expression of MTF1, which in turn, promotes MT expression.	MTF1 binds to the MRE of CTR1B to promote its transcription and expression, which introduces copper, and maintains copper homeostasis when copper is depleted.	[28]
p53	p53 might promote cuproptosis by inhibiting glycolysis, and enhancing mitochondrial metabolism.	Cancer	p53 regulates FDXR that encodes a ferredoxin reductase responsible for electron transport from NADPH to FDX1/2, and subsequently to cytochrome P450 for Fe-S cluster biogenesis (p53 induces the expression of Fe-S cluster scaffold components ISCU and FXN).	Tumor suppressor microprotein miPEP133, which is encoded by the primary transcript of miR-34a activated by p53, was found to interact with HSPA9 to impair its function in the mitochondria/HSPA9 serves as a chaperone protein for the Fe-S cluster assembly.	[198]
Disulfiram	Disulfiram is a copper carrier, which combines with copper to promote cell death.	Cancer	Disulfiram combined with Cu^{2+} promoted ROS production, activated the p38 pathways, and inhibited the NF-kB signaling pathway, in order to induce cancer cell death.	The disulfiram-Cu^{2+} complex damaged cancer cells by restraining proteasome activity, and increasing ubiquitin-protein interactions.	[256]

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Regulators of signaling pathways or molecules targeted by Cu	Function	Disease	Possible signaling pathway	Other findings	References
COMMD10	COMMD10 is a cancer suppressor and copper metabolism regulator.	Cancer	COMMD10 inhibits the HIF1a/CP positive feedback loop to enhance radiosensitivity by disrupting Cu-Fe balance.	COMMD10, HIF1α, CP, and SLC7A11 might be potential new targets and predictive biomarkers of radioresistant HCC.	[223]
NRF2	The NRF2 is responsible for the regulation of antioxidant response, and plays a critical role in mitigating ferroptosis.	Cancer	DSF/Cu dramatically activates the phosphorylation of p62, which facilitates the competitive binding of Keap1, thereby prolonging the half-life of NRF2.	DSF/Cu could strengthen the cytotoxicity of sorafenib, and arrest tumor growth, both *in vitro* and *in vivo*, by simultaneously inhibiting the signal pathway of NRF2 and MAPK kinase.	[224]
GPX4	GPX4 plays a master role in blocking ferroptosis by eliminating phospholipid hydroperoxides.	Cancer	Copper induces the macroautophagic degradation of GPX4 to drive ferroptosis and TAX1BP1, and this acts as an autophagic receptor for GPX4 degradation and subsequent ferroptosis in response to copper stress.	Exogenous copper increases GPX4 ubiquitination and the formation of GPX4 aggregates by directly binding to GPX4 protein cysteines C107 and C148.	[225]
HMGB1	HMGB1, a damage-associated molecular pattern, is released by cuproptotic cells to initiate inflammation.		Copper accumulation-induced ATP depletion activates AMPK to promote HMGB1 phosphorylation, resulting in increased DAMP extracellular release.	In HMGB1-deficient cuproptotic cells, AGER-dependent inflammatory cytokine production is greatly reduced.	[199]
GNAQ	GNAQ plays an important role in GPCR signaling.	Uveal melanoma	In GNAQ mutated cells, Cu-ES produces ROS. Subsequently, this promotes YAP phosphorylation, and inhibits its nuclear accumulation. The inactivation of YAP downregulates the expression of SNAI2, which in turn, suppresses the migration of UM cells.		[202]

Cu: copper; mTOR: mammalian target of rapamycin; COG: conserved oligomeric Golgi; Nedd4l: NEDD4 like E3 ubiquitin protein ligase; MT: metallothionein; MRE: metal responsive element; COMMD10: copper metabolism MURR1 domain 10; NRF2: nuclear factor erythroid 2-related factor 2; TAX1BP1: Tax1 binding protein 1; GPX4: glutathione peroxidase 4; HMGB1: high-mobility group box 1; ATP: adenosine triphosphate; AMPK: AMP-activated protein kinase; AGER (RAGE): advanced glycosylation end product-specific receptor; GNAQ: guanine nucleotide-binding protein G(q)

activation of autophagy can protect hepatocytes from copper-induced death[195]. The interaction between cuproptosis and ferroptosis generally occurs after the imbalance of copper homeostasis and iron homeostasis. COMMD10, which is a copper homeostatic regulator, can regulate the occurrence of ferroptosis[223]. Present research on these cell deaths is focused on cancer. The disulfiram-Cu complex triggers ferroptosis, and copper stimulates ferroptosis by inducing the degradation of GPX4, which is a key regulator in autophagy[225]. High levels of ROS, oxidative stress, and inflammation may be the common features of these cell deaths. Based on its similarity and previous studies, it is reasonable to presume that there is a regulatory complex that acts as a common mechanism of cell death, which is similar to the PANoptosome in PANoptosis[244].

Given the important functions of microglia and astrocytes in the nervous system[245], it is critical to research the function of copper on microglia and astrocytes in disease states associated with the recognition and clearance of abnormal Aβ peptides and tau proteins by the immune system[246]. The role of microglia and reactive astrocyte proliferation in copper toxicity response remains to be elucidated. Guidelines for astrocyte-mediated intervention and unstable copper ions based on neuronal activity in astrocyte-neuron metabolism have been proposed[247]. Although the focus of the present research was on the nervous system, it is important to recognize the heterogeneity of neurodegenerative diseases, with detrimental effects observed in the whole body. Copper intake can be controlled through precision medicine, and individualized treatment plans based on genetics, diet, lifestyle, culture, and access to resources, given the long-term pathological effects of abnormal copper metabolism in AD.

Early detection through advanced approaches, and treatment with various strategies for neurodegenerative diseases are very important. For disease investigation, neurons derived from human embryonic stem cells have been shown to faithfully recapitulate the specific neural development of an individual[248]. Single-molecule localization microscopy allows for the formation, assembly, and dissociation of protein complexes in real time[249]. The use of CRISPR gene editing *in vitro*[250] can contribute to the study of cellular functions and signaling pathways. In addition, studies should be based on complex models *in vivo*. Novel research directions are ideally guided by one animal model with the same category of neurodegenerative pathology, in order to compare the similarities and differences of various diseases. Copper chelators and ionophores represent the main drug therapies for impaired copper homeostasis in the brain[251]. Chelators should be designed to safely cross the BBB, and target specific neural networks, without interfering with the normal physiological functions of peripheral nerves and the brain. Furthermore, a "combination drug-multitarget" therapeutic strategy would be appropriate for controlling AD. The present strategy of medicinal chemists in combating AD is to design and investigate multifunctional drugs with anti-Aβ effects, acetylcholinesterase inhibition, antioxidants, and metal chelator activities[252]. Chinese herbal medicines have exhibited promising therapeutic potential[253]. China has constructed a large library of traditional medicines. One of the ancient anticancer drugs recorded is curcumin, which is derived from the rhizomes of plants. Curcumin has been shown to be protective against AD[254]. Furthermore, flavonoids, which are important antioxidants and signaling molecules, may slow the disease process, and improve neurocognition in AD patients[255]. Pharmacological treatments may need to consider the following: (1) the use of multifunctional compounds or single-molecule drugs to delay the onset of the disease; (2) the ideal dose when administering the drug; (3) the optimal time of administration.

5 CONCLUSION

Copper is involved in key biochemical pathways, such as mitochondrial respiration, antioxidant mechanisms, and death initiation. Excess copper in cells induces redox activity, and generates hydroxyl radicals, which induce oxidative stress and cellular damage. Copper has been implicated in deleterious protein aggregation or neuroinflammation, leading to the progression of various diseases within the nervous system. Therefore, intracellular copper must be tightly regulated to maintain copper homeostasis in the body, especially in the brain. Copper homeostasis has been identified as a key target for delineating the link between astrocyte copper metabolism and neuronal metabolism, and for the treatment of neurodegenerative diseases. It is critical to ensure that copper is ingested in appropriate amounts to support normal living activities, but without the deleterious effects of copper toxicity. The mechanisms of copper homeostasis provide potential therapeutic insights into genetic disorders of copper metabolism and neurodegenerative diseases, such as Wilson disease, Menkes disease, AD, PD, ALS, and HD. However, a number of unanswered questions on the cellular mechanisms of copper uptake, transport, and utilization remain. Ongoing studies that investigate copper transporters, metallochaperones, and copper proteases in a wide range of pathological conditions continue to broaden the field of existing treatments, and provide additional therapeutic targets.

Availability of Supporting Data

The table data were obtained from ClinicalTrials.gov, which is a public database. Ethics approval was obtained for patients involved in the database. Users can download relevant data for free for research, and publish relevant articles.

Conflict of Interest Statement

The authors declare no conflicts of interest. Author Kun XIONG is a member of the Young Editorial Board for Current Medical Science. The study was handled by another editor, and has undergone a rigorous peer review process. Author Kun XIONG was not involved in the journal's review of or decision related to the manuscript.

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