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Review

Cuproptosis and Aging: Molecular Mechanisms and Therapeutic Implications

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ABSTRACT: With the accelerated pace of global population aging, the number of people suffering from age-related diseases is increasing, posing a serious threat to human health and well-being. Age-related diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), hypertension, atherosclerosis, type 2 diabetes (T2DM), and osteoporosis (OP), are often characterized by physiological function decline and metabolic disorders caused by aging, among which dysregulation of metal ion homeostasis, especially copper homeostasis imbalance, has emerged as a major factor. Copper is an essential enzymatic cofactor whose homeostasis is tightly regulated at systemic, cellular, and subcellular levels. However, during the aging process, the balance between copper uptake and efflux becomes compromised, alongside a reduction in cellular copper-buffering capacity. These alterations may lead to the loss of copper homeostasis and induce cuproptosis, which is a recently elucidated form of regulated cell death (RCD) triggered by copper overload and disrupts mitochondrial metabolism by promoting the aggregation of lipoylated proteins in the tricarboxylic acid cycle (TCA) and destabilizing respiratory chain complexes. Copper homeostasis imbalance and the resulting cuproptosis accelerate the aging process by promoting molecular mechanisms, including telomere attrition, mitochondrial dysfunction, oxidative stress, proteostasis imbalance, epigenetic changes, and chronic inflammation. This review focuses on the reciprocal interactions between aging and copper homeostasis: it elucidates how aging impairs copper homeostatic regulation, and how dysregulated copper metabolism and subsequent cuproptosis accelerate aging and exacerbate age-related diseases. Furthermore, it explores potential therapeutic strategies targeting copper homeostasis and cuproptosis to treat age-related diseases.

Keywords: cuproptosis, copper, aging

1. Introduction

Aging is a multifaceted biological process characterized by the gradual deterioration of physiological functions, resulting in a higher risk of chronic diseases and mortality worldwide [1]. By 2050, the United Nations predicts that the global population of those aged 60 and older will double to 2.1 billion, while those aged 80 and above will triple to 426 million [2]. This demographic shift signals a sustained rise in the occurrence of age-related diseases, consequently imposing substantial economic burdens and increased pressures on healthcare systems [3]. Age-related diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), hypertension, atherosclerosis, type 2 diabetes mellitus (T2DM), and osteoporosis (OP), pose a significant challenge to global public health systems [4, 5]. The core molecular mechanisms of aging include the accumulation of macromolecular damage,

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mitochondrial dysfunction, impaired proteostasis, epigenetic alterations, and chronic inflammation, all of which contribute to cellular senescence and tissue degeneration [6]. Among the various mechanisms implicated in aging, regulated cell death (RCD) has emerged as critical players in mediating age-associated cellular damage and organ dysfunction [7]. Recently, a novel form of copper-dependent RCD, termed cuproptosis, has been identified, revealing a previously unrecognized link between copper homeostasis and cellular viability. Cuproptosis is a distinct, copper-dependent mode of cell death characterized by its disruption of the mitochondrial respiratory chain. This process is initiated by the excessive accumulation of copper ions in cells, causing the oligomerization of lipoylated proteins in the tricarboxylic acid cycle (TCA), which triggers cellular proteotoxic stress. Ferredoxin 1 (FDX1) has been identified as a key upstream regulator that drives cuproptosis by modulating protein lipoylation [8]. The interplay between RCD and autophagy in tumor biology is well documented [9-12]. Building on this foundation, the recent discovery of cuproptosis has identified a distinct pathway increasingly implicated in tumor initiation, progression, and the pathogenesis of various inflammatory and neoplastic diseases [13-15]. The discovery of cuproptosis offers a novel perspective for investigating age-related diseases. Given that copper is an essential enzymatic cofactor critical for mitochondrial respiration, antioxidant defense, and neurotransmitter synthesis, its dyshomeostasis during aging could have profound implications for cellular integrity and tissue function [16]. Aging is often accompanied by alterations in metal ion distribution and accumulation, including copper [17]. Copper homeostasis imbalance and cuproptosis may accelerate the aging process and the onset of age-related diseases through oxidative stress, mitochondrial damage, and cellular loss. This review aims to comprehensively analyse the potential role of copper homeostasis imbalance and cuproptosis in the core molecular mechanisms of aging. We will explore how aging leads to copper homeostasis imbalance, and how copper homeostasis imbalance and cuproptosis contributes to telomere attrition, mitochondrial dysfunction, impaired proteostasis, epigenetic alterations, and chronic inflammation in aging (Fig. 1).

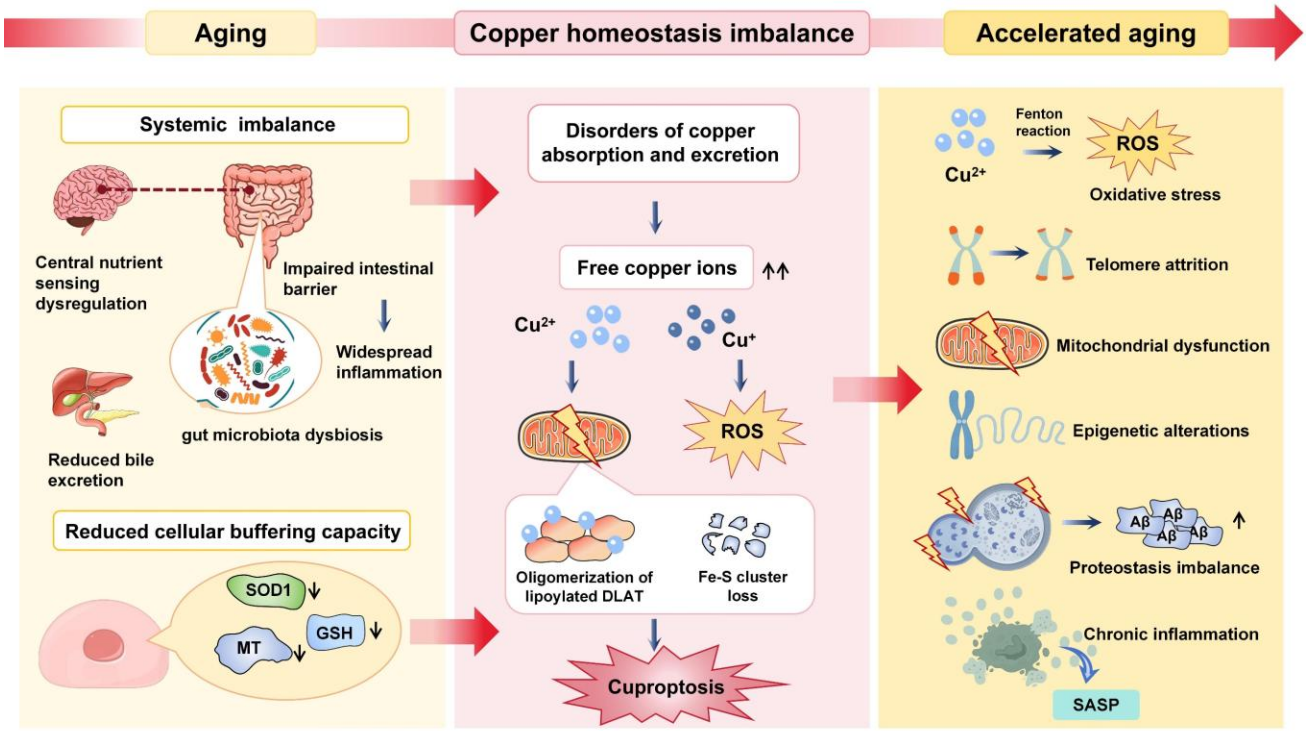

Figure 1. The interplay between copper homeostasis, cuproptosis, and aging mechanisms. The diagram illustrates that age-related systemic imbalances (e.g., impaired nutrient sensing, intestinal barrier dysfunction, and inflammation) together with reduced cellular buffering capacity (reflected in lower GSH, SOD1, and MT protein levels) disrupt copper absorption and excretion. This leads to an accumulation of free copper ions. Cu^{+} potently catalyzes ROS production, driving oxidative stress. Concurrently, Cu^{2+} promotes the oligomerization of lipoylated DLAT proteins and disrupts the stability of Fe-S cluster proteins within mitochondria, ultimately triggering cuproptosis. Copper homeostasis imbalances and cuproptosis, in turn, accelerate aging by exacerbating its core hallmarks such as enhanced oxidative stress, telomere attrition, mitochondrial dysfunction, epigenetic alterations, proteostasis imbalance, and chronic inflammation.

Furthermore, we will discuss emerging therapeutic strategies targeting cuproptosis, such as copper chelation, cuproptosis inhibitors, natural medicines, nanomedicine and gene therapy, which show potential for reducing age-related decline and enhancing healthy aging. By elucidating the intricate interplay between copper metabolism and cellular senescence, this review seeks to provide new insights into the potential of cuproptosis as a therapeutic target for aging and associated pathologies.

2. Copper homeostasis

2.1 Systemic copper homeostasis

Copper is distributed throughout the body, with the highest concentrations found in the kidneys, followed by the liver and brain, while the largest total stores are in bone and skeletal muscle [18]. Dietary copper is primarily absorbed in the stomach and duodenum, subsequently entering hepatic circulation via the portal vein. Hepatocytes then sequester and store most of this newly absorbed copper. Copper excretion mainly occurs through bile and pancreatic secretions into the gastrointestinal tract, with minimal contributions from saliva and gastric fluid [19, 20]. Dietary copper typically presents in the form of Cu^{2+}. Following reduction to Cu^{+} by metal reductase enzymes, it enters the intestinal epithelial cells via the copper transporter 1 (CTR1) located on the cell membrane of these cells. In addition, Cu^{2+} can enter the cells via divalent metal transporter 1 (DMT1) and subsequently be reduced to Cu^{+} by endogenous bioreductase enzymes and vitamin C [21]. Cytoplasmic copper is regulated by metallothionein (MT) and antioxidant 1 (ATOX1), which function in buffering and release. These proteins deliver copper to ATPase copper transporting alpha (ATP7A) for transport to the trans-Golgi network (TGN). Under elevated copper conditions, ATP7A relocates to the basolateral membrane to facilitate copper efflux into circulation [22]. As part of the buffering system of most mammals, blood albumin has a number of high-affinity binding sites that sequester excess copper [23]. Copper in serum accounts for about 5% of total copper and is mainly bound to ceruloplasmin (CP), and transported to specific tissues and organs, such as the brain, heart, muscles and bones for utilization [24, 25]. While liver-secreted and synthesised CP cannot cross the blood-brain barrier (BBB), CP within the brain is synthesised by astrocytes, where it is anchored to the plasma membrane and linked to glycosylphosphatidylinositol [26]. Copper penetrates the interstitial fluid (ISF) of the brain via two main pathways: the BBB and the blood-cerebrospinal fluid barrier (BCB). ATP7A, CTR1 and ATPase copper transporting beta (ATP7B) are major copper transport proteins, and the BCB serves as the main pathway for the elimination of excess copper from the brain [27, 28]. Astrocytes import interstitial copper through membrane-bound CTR1 transporters and export it via ATP7A. Within the BCB, ATP7B exhibits slower kinetics than ATP7A, resulting in a slower rate of copper entry into the cerebrospinal fluid (CSF) relative to the rate of choroid plexus copper uptake, thereby aiding in the segregation of copper from the choroid plexus. In contrast, the faster movement of ATP7A in the basolateral membrane aids in swiftly eliminating surplus copper from the brain into the bloodstream [29]. At high copper intake levels, copper absorption decreases while copper excretion increases; conversely, at low copper intake levels, endogenous copper excretion in bile diminishes and copper absorption increases [30].

2.2 Copper homeostasis in cells

Copper enters cells primarily as Cu^{+} via the CTR1 transporter, where it is distributed into stable and unstable pools. The unstable, labile copper pool, often termed “free copper”, is highly reactive and can participate in Fenton reactions, generating harmful reactive oxygen species (ROS). To mitigate toxicity, free copper is buffered by binding to MT1/2, ATOX1, and glutathione (GSH) [31, 32]. Copper bound to the copper chaperone for superoxide dismutase (CCS) is transferred to Copper/Zinc superoxide dismutase (SOD1) to participate in electron chain transfer for antioxidant protection. The copper chaperone ATOX1 delivers copper to the Cu-ATPases, ATP7A/B, on the trans Golgi network membrane before being pumped into the TGN lumen [33]. In the secretory pathway, under conditions of elevated cellular copper levels, Cu ATP7A/B translocate from the TGN to the vicinity of the cell membrane. Cytoplasmic copper-bound ATOX1 is also transferred to ATP7A/B. Following phosphorylation by a kinase, ATP7A/B travels to the cell membrane of vesicles, facilitating fusion and release from the cell via ATP7A/B. Copper from the stable pool bound to a copper ligand (CuL) is then exchanged in the mitochondrial intermembrane space (IMS) to cytochrome c oxidase 17 (COX17) [34], a protein essential for mitochondrial respiration [35]. COX17 transfers the copper to COX11 and synthesis of cytochrome c oxidase 1 (SCO1) in the mitochondrial matrix, initiating two pathways, with COX11 transferring copper to COX1's copper-binding site, CuB, in the COX1 to COX1, and SCO1 providing copper to COX2 at the copper-binding site CuA of COX2. COX1 and COX2 are jointly involved in the biological process of cytochrome c oxidase (CCO) [36]. A portion of copper still bound to CuL enters the mitochondria and binds to CCS at the IMS and subsequently to SOD1,

which, in conjunction with Zn, forms SOD1, essential for redox processes [37, 38] (Fig. 2).

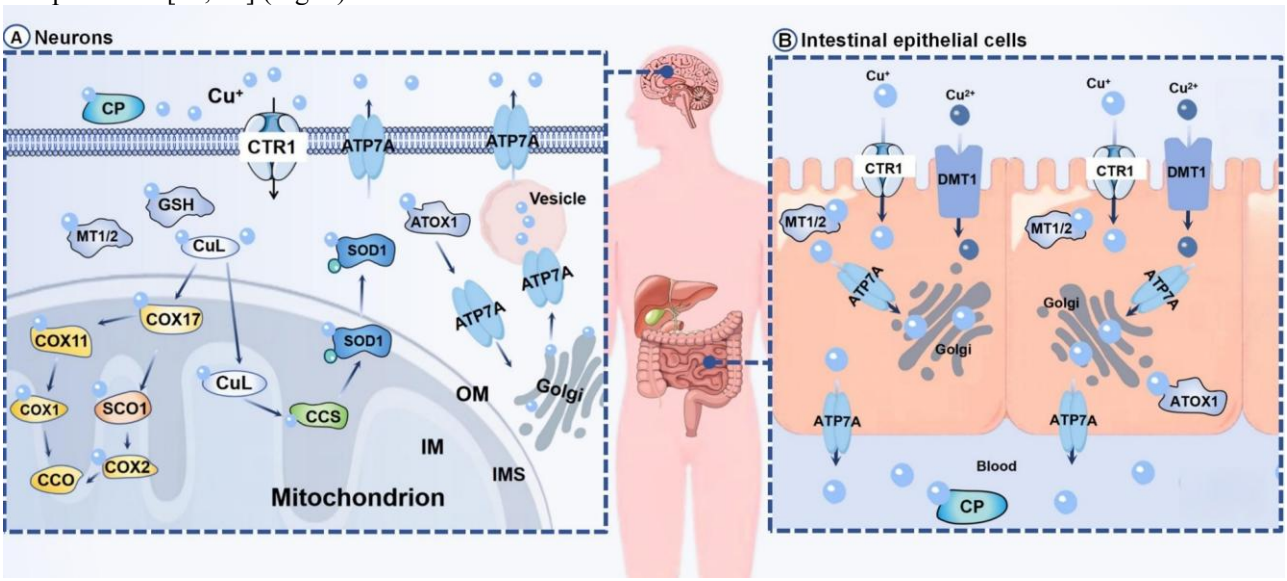

Figure 2. Copper metabolism at the molecular level. (A) Extracellular copper mainly binds to CP, and brain cells uptake Cu^{+} into the cell through CTR1. MT1/2, GSH, and ATOX1 act as buffer systems to chelate excess Cu^{+}, which is released when necessary. Copper ligands (CuL) deliver Cu^{+} into mitochondria, while COX17 carries Cu^{+} to COX11 and SCO1, and then to COX1 and COX2, participating in mitochondrial respiration. ATP7A transports Cu^{+} to the Golgi apparatus for protein synthesis, and excess Cu^{+} is transported to the vicinity of the cell membrane through vesicles, where it is expelled from the cell through ATP7A on the membrane. (B) Cu^{+} and Cu^{2+} enter the intestinal epithelium through the uptake of CTR1 and DMT1, respectively. Intracellularly, ATP7A transports copper into the Golgi apparatus and excretes excess copper out of the cell. MT1/2 and ATOX1 act as buffer systems, chelating excess copper and being released when necessary.

3. Cuproptosis

Cuproptosis is a novel mode of cell death induced by copper ions [39]. In cancer therapy, the copper ionophore elesclomol (ES) facilitates cellular copper uptake and induces cell death in a Cu^{+}-dependent manner. Notably, ES alone is non-toxic, underscoring the essential role of copper overload. Cuproptosis is regulated by mitochondrial FDX1-mediated protein lipoylation. Lipoylation, a mitochondria-specific post-translational modification where mitochondrial lipoic acid covalently binds to specific amino acid residues of lipoylated proteins [40, 41], including DBT (branched-chain α-ketoacid dehydrogenase complex component), GCSH (glycine cleavage system protein H), DLST (α-ketoglutarate dehydrogenase complex component), and DLAT (the E2 subunit of the pyruvate dehydrogenase complex), exhibit high affinity for copper. FDX1 also facilitates the reduction of copper ions and the conversion of Cu^{2+} to Cu^{+}. Excess copper prompts oligomerization of these modified proteins, particularly DLAT, disrupting TCA cycle and leading to proteotoxic stress. Concurrently, the FDX1-dependent iron-sulfur (Fe-S) cluster proteins are lost. These events collectively trigger cell death via proteotoxic stress. It acts in the mitochondrial respiratory pathway, so cells with sensitive mitochondrial respiration are more susceptible to induced cuproptosis. This is not the same as the copper-induced cell death that was thought in the past, where it was thought that increased ROS produced by copper overproduction led to oxidative stress causing cell death, however, the cytotoxic effect caused by ES-Cu overload was not eliminated with the ROS inhibitor, N-acetylcysteine (NAC), which suggests that the form of cuproptosis is not oxidative stress (Fig. 3). The key regulatory genes for cuproptosis included *FDX1*, *LIAS*, *DLD*, *PDHA1*, *PDHB*, and *DLAT*. Additionally, the three negative regulators of cuproptosis include glutaminase (GLS), cyclin-dependent kinase inhibitor 2A (CDKN2A), and metal regulatory transcription factor 1 (MTF1) [8]. The morphology of cells undergoing cuproptosis remains poorly understood. In the context of pathological copper overload, such as observed in Wilson's disease (WD) (characterized by chronic copper overload), copper-overload cells exhibit condensed mitochondria with separated inner and outer membranes, normal outer mitochondrial membranes, narrowed and distorted cristae interstitial space, increased matrix density or replacement by large vacuoles, and mitochondrial inclusions of different sizes [42, 43]. Copper chelators can rescue cells from cuproptosis [44].

The tumour suppressor P53 is widely thought to be associated with many RCD pathways, such as apoptosis and ferroptosis. Recent evidence suggests that P53 increases susceptibility to cuproptosis by inhibiting glucose metabolism, resulting in the accumulation of pyruvate, which drives a shift towards mitochondrial metabolism. Besides, P53 promotes cuproptosis by facilitating dephosphorylation, which converts pyruvate to acetyl coenzyme A, activating the pyruvate dehydrogenase (PDH) complex (a key component of cuproptosis), as well as glutamine, and enhancing mitochondrial metabolism [45]. P53 is activated during cellular senescence, inducing cell cycle arrest, which may render cells more susceptible to cuproptosis [46, 47].

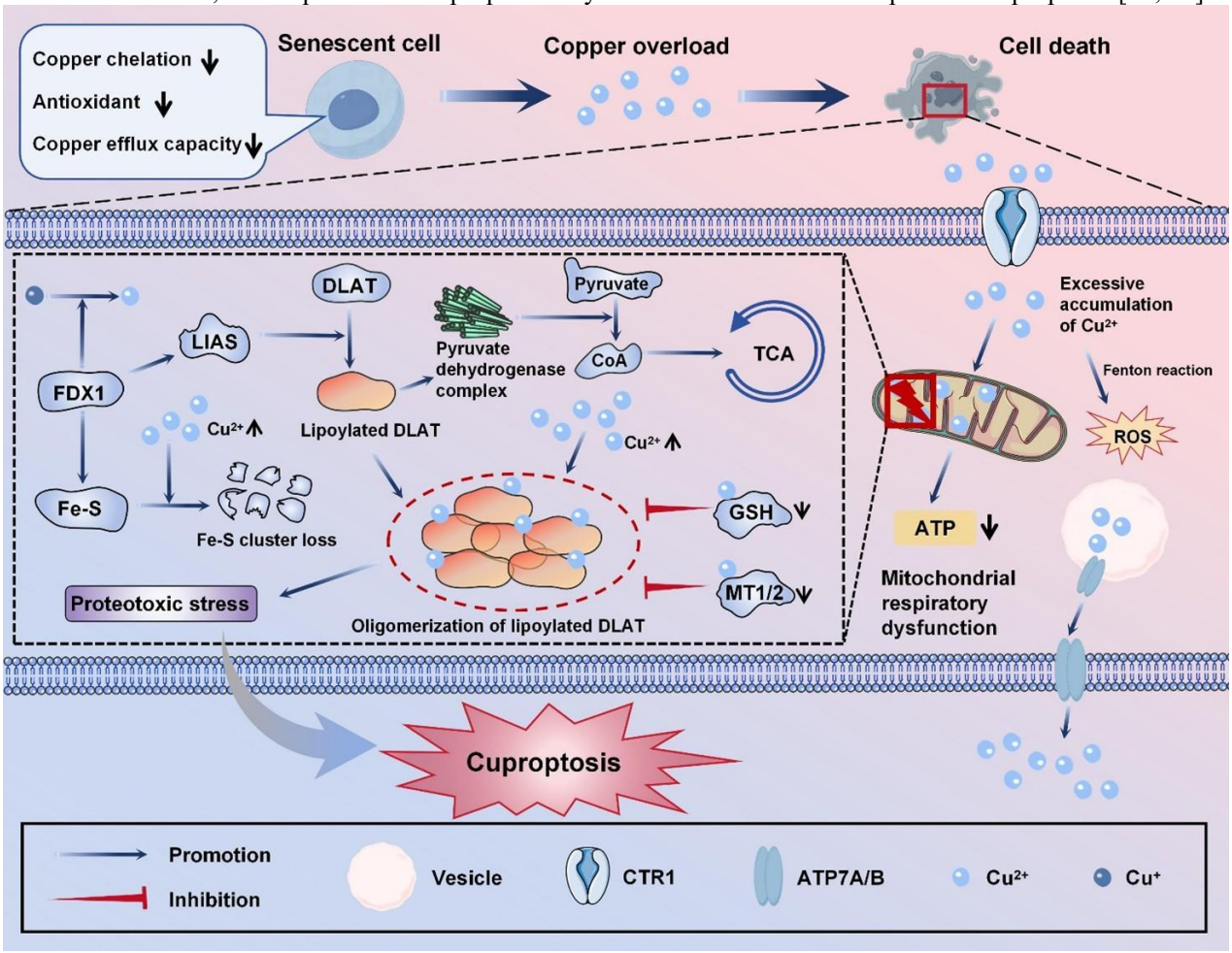

Figure 3. Mechanism diagram of cuproptosis. Endogenous chelators like GSH and MT1/2 protect cells by sequestering copper to prevent toxic aggregation. In senescent cells, the decline in copper-chelation, antioxidant, and copper efflux capacities leads to cellular copper overload and consequent cell death. Disrupted copper homeostasis results in the accumulation of free Cu^{2+}, which catalyzes ROS production via Fenton reaction and impairs the mitochondrial respiratory chain, reducing ATP production. To manage excess copper, cells utilize vesicular trafficking to sequester copper, with ATP7A mediating its export from the cell. Within mitochondria, FDX1 regulates protein lipoylation. Through LIAS, DLAT becomes lipoylated and integrates into the pyruvate dehydrogenase complex to support the TCA cycle and ATP generation. FDX1 also maintains the stability of Fe-S cluster proteins. Under copper overload, lipoylated DLAT forms oligomers, inducing proteotoxic stress, while Fe-S cluster proteins become destabilized. These cumulative defects ultimately trigger cuproptosis.

FDX1 is a highly conserved Fe-S cluster protein located in the mitochondrial matrix, and it plays a role in various metabolic processes, including steroidogenesis, bile acid synthesis, Fe-S cluster biogenesis, and vitamin D synthesis [48]. The expression of FDX1 declines with ovarian aging, suggesting it may serve as a promising biomarker for ovarian aging. This reduction in FDX1 expression disrupts mitochondrial energy metabolism, leading to excessive ROS production and inducing oxidative stress-mediated cell death [49].

DLST, as a key enzyme in the TCA, directly influences mitochondrial oxidative phosphorylation function, determining cellular metabolic stability and the severity of senescence phenotypes. Depletion of DLST in senescent cells exacerbates growth arrest and enhances senescence-associated β-galactosidase activity, triggering

a metabolic reprogramming of cellular energy pathways. This shift from oxidative phosphorylation towards glycolysis manifests as increased extracellular acidification rates and lactate production. While this metabolic transition constitutes an adaptive response enabling cellular survival under oxidative stress, it is also intrinsically linked to the senescence process [50]. The key genes of cuproptosis, along with their links to aging and age-related diseases, are summarized in Table 1.

Table 1. Key genes involved in cuproptosis and their relevance to aging and age-related diseases.

Genes	Roles in cuproptosis	Relevance to aging	Age-related diseases	Refs.
FDX1	Upstream regulator; reduces Cu^{2+} to Cu^{+}, mediates protein lipoylation and Fe-S cluster biogenesis.	Expression declines with ovarian aging; loss may disrupt mitochondrial metabolism and increase oxidative stress.	Upregulated in AD neurons and aged mouse and Bama minipig myocardial ischemia-reperfusion (MIR) models, promoting cuproptosis	[49, 51, 52]
LIAS	Encodes lipoic acid synthase; critical for the protein lipoylation targeted during cuproptosis.	Not explicitly studied in aging, but its product α-lipoic acid levels decline with age, affecting mitochondrial function.	Overexpression of LIAS promotes regulatory T cell (Treg) proliferation, reduces vascular inflammatory responses and alleviates atherosclerosis	[53, 54]
DLAT	Lipoylated component of the PDH complex; oligomerization under copper overload triggers proteotoxic stress.	Sirtuin4 (SIRT4) expression or activity declines during aging, which can lead to excessive DLAT lipoylation and subsequent metabolic dysfunction.	Upregulated in age-related hearing loss mouse models	[55, 56]
DLST	Lipoylated component of the α-ketoglutarate dehydrogenase complex; a direct target for copper binding and oligomerization.	Senescence-induced long noncoding RNA (sin-lncRNA) directly interacts with DLST, maintaining its mitochondrial localisation and regulating metabolism in senescent cells.	Upregulated in age-related hearing loss mouse models	[50, 56]
ATP7A	Regulation of copper ion transport.	Functional decline in aged mouse models and aged Bama minipig models, reducing copper efflux and triggering cuproptosis.	In aged mouse and Bama minipig MIR models, reduced membrane-bound ATP7A impairs copper efflux, triggering cuproptosis.	[52, 57]
CTR1 (SLC31A1)	High-affinity copper ion transporter on the cell membrane.	Elevated in senescent cells	Upregulated in the hippocampus of AD mouse models.	[57, 58]
MTF1	Metal-regulatory transcription factor; a negative regulator of cuproptosis.	Alleviates oxidative stress associated with metal toxicity and extends lifespan	Significantly decreased in male T2DM mouse models.	[59, 60]
CDKN2A	A tumor suppressor gene; negative regulator of cuproptosis.	A canonical marker of cellular senescence; expression increases with age.	P16, encoded by the CDKN2A gene, is elevated in AD neurons.	[61, 62]
GLS	Participates in glutaminolysis; a negative regulator of cuproptosis.	Suppression of glutaminolysis promotes senescent cell clearance.	Suppression of GLS activity significantly ameliorates neuroinflammation in AD mouse models.	[63, 64]

FDX1, Ferredoxin 1; Fe-S, Iron-sulfur; AD, Alzheimer's disease; LIAS, Lipoic acid synthetase; DLAT, Dihydrolipolipoamide acetyltransferase; PDH, Pyruvate dehydrogenase; DLST, Dihydrolipoamide S-succinyltransferase; ATP7A, ATPase copper transporting alpha; MIR, myocardial ischemia-reperfusion; CTR1, Copper transporter 1; MTF1, Metal regulatory transcription factor 1; T2DM, type 2 diabetes; CDKN2A, Cyclin-Dependent Kinase Inhibitor 2A; GLS, Glutaminase.

4. The impacts of aging on copper homeostasis

4.1 Systemic copper homeostasis imbalance in aging

Aging is intricately linked to the dysregulation of essential metal homeostasis, with copper emerging as a particularly critical element due to its dual role as a vital cofactor and a potential source of oxidative damage. The precise mechanisms underlying age-associated systemic copper accumulation and its contribution to the pathogenesis of age-related diseases, remain an area of intense investigation. Increasing evidence indicates that this phenomenon is not driven by a single defect but rather arises from a complex interplay between central nutrient

sensing, hepatic handling, biliary excretion, and gut-brain axis communication.

Copper uptake in the intestine is regulated by nutrient sensing within the central nervous system (CNS). Aging disrupts nutrient sensing functions, subsequently downregulating the expression levels of copper reductases six-transmembrane epithelial antigen of the prostate 2 (STEAP2) and STEAP3, along with the copper transporter CTR1 in intestinal tissues. Theoretically, these changes could potentially inhibit copper absorption efficiency [65]. However, earlier tracer studies using ^{65}Cu in aged rats revealed no significant difference in copper absorption rates compared to young rats, while serum copper levels remained markedly elevated [66]. This phenomenon suggests that, rather than enhanced absorption, compromised copper excretion may play a more critical role in driving age-related systemic copper accumulation.

Bile excretion represents the primary route for the elimination of excess copper from the human body. Although no direct evidence has established that aging reduces copper excretion via bile, recent studies indicate a significant decline in overall biliary excretion rates among the elderly [67]. This age-related reduction in biliary output may indirectly decrease the total copper excreted, thereby contributing to systemic copper accumulation. As the central organ for copper metabolism, the liver plays a critical role in maintaining copper homeostasis. Studies utilizing biocompatible labile copper probes in aged mice have demonstrated copper accumulation in the liver, along with a marked increase in the labile copper pool with advancing age [68]. Such hepatic copper retention, coupled with elevated levels of labile copper, not only disrupts hepatic metabolic function and accelerates hepatic aging, but also further elevate plasma copper levels, establishing a vicious cycle of impaired excretion, hepatic copper overload, and increased systemic copper burden.

Emerging evidence underscores a strong link between aging and gut microbiota dysbiosis, with such microbial alterations indirectly perturbing systemic copper homeostasis through the induction of chronic inflammation. Age-related changes in the gut microbiome are characterized by a decreased Firmicutes/Bacteroidetes ratio and a reduction in beneficial microbes, including Bifidobacterium [69, 70]. This dysbiosis promotes two key pathological processes: (1) Impairment of the intestinal barrier: disruption of epithelial tight junctions increases intestinal permeability, facilitating the translocation of pro-inflammatory luminal molecules such as lipopolysaccharide (LPS) into systemic circulation, thereby initiating widespread inflammation; (2) Expansion of pro-inflammatory microbiota: metabolic shifts, such as decreased short-chain fatty acid production and increased endotoxin release, further amplify systemic inflammatory signalling, sustaining a state of chronic low-grade inflammation [71]. This age-associated inflammatory milieu disrupts cellular and systemic copper homeostasis. Concurrently, inflammation-driven immunosenescence has been implicated in the pathogenesis of neurodegenerative diseases, including AD and PD [72-74]. Growing evidence suggests that dysregulated copper homeostasis acts as an upstream modulator linking chronic inflammation and neurodegeneration. The proposed cascade- inflammation-copper dysregulation-neuroinflammation - neuronal damage, may constitute a critical mechanism underlying age-related neurodegenerative diseases.

During physiological aging, changes in copper influx into the brain exhibit a distinct dynamic pattern, initially increasing and subsequently decreasing with advancing age. A longitudinal study utilised $^{64}CuCl_2$-PET/CT technology in C57BL/6 mice, measuring ^{64}Cu radioactivity changes in the brain and whole body at 5, 13, and 26 months of age following oral administration of $^{64}CuCl_2$ as a tracer. At 13 months of age, ^{64}Cu uptake in the mouse brain was significantly higher than at 5 months, subsequently decreasing by 26 months [75]. This indicates that during the aging process, the influx of copper into the brain increases during middle age but diminishes in old age. In addition, a study employing ventriculo-cisternal perfusion experiments revealed that ^{64}Cu radioactivity in the CSF of 18-month-old aged rats was considerably higher than in young and adult rats, indicating the copper clearance at the BCB is reduced during aging. Moreover, the expression of copper transporters in the choroid plexus exhibits age-specific patterns, CTR1 expression was reduced with diminished distribution, while the weaker Cu uptake protein DMT1 expression showed no significant change. ATP7A exhibited apical/basal aggregation, and ATP7B expression was slightly elevated, suggesting diminished copper clearance capacity in the choroid plexus [76]. Compared with young individuals, aged choroidal endothelial cells exhibit a significant increase in the thickness of collagen IV deposits, which further perturbs the structural integrity of these endothelial cells. Notably, the choroid serves as a critical anatomical site for mediating copper efflux from the brain, and choroidal tissue from aged individuals shows reduced expression of CTR1. Concomitantly, the BCB, a pivotal structure for maintaining CSF homeostasis, undergoes age-related downregulation of Zonula Occludens-1 and Claudin-12, two genes that encode core components of tight junctions. The combined effects of CTR1 reduction and tight junction gene dysregulation directly impair the structural and functional integrity of the BCB [76], leading to a marked decline in copper clearance efficiency from the

CSF, thereby promoting the progressive accumulation of copper, exacerbating oxidative stress and inflammatory response within the aging brain.

4.2 Reduced cellular buffering capacity

Cellular antioxidant capacity declines significantly during aging. Astrocytes play a key role in regulating copper homeostasis through the sequestration and storage of copper. With advancing age, periventricular astrocytes exhibit both a decline in density and compromised functionality, which collectively impair the brain's copper-buffering capacity, resulting in dysregulation of cerebral copper homeostasis and subsequent accumulation of neurotoxic copper species [77]. Such redox dysregulation is also observed in other cell types. The redox homeostasis of red blood cells is typically compromised during the aging process and promotes thrombus formation [78]. Aging macrophages exhibit impaired ROS clearance and increased oxidative stress, thereby promoting inflammatory damage to hepatocytes [79]. Concurrently, GSH possesses significant copper-chelating capacity and is a vital substance for antioxidant defence, detoxification, and the mitigation of aging processes [80, 81]. GSH depletion occurs with aging, largely attributed to compromised biosynthesis, ultimately inducing cellular dysfunction [82]. Notably, elderly populations demonstrate reduced cerebral GSH levels, this essential antioxidant supports mitochondrial function and facilitates the clearance of misfolded proteins [83, 84]. Chelating free copper is crucial to prevent ROS production and mitochondrial damage, as GSH deficiency promotes free copper buildup in cells, thereby facilitating deleterious oxidative processes. Copper catalyzes the oxidation of dopamine (DA), generating toxic quinones and ROS such as superoxide anions ($\cdot O_2^-$) and hydroxyl radicals, which drive oxidative damage in dopaminergic neurons [85]. The decline in antioxidant capacity also manifests as reduced activity or expression of key enzymes and regulators. The activity of key enzymes in GSH synthesis, such as glutamate-cysteine ligase (GCL) and glutathione synthase (GS), declines with age, leading to reduced GSH synthesis [86]. Significant reductions in GSH levels were observed across multiple brain regions (cortex, striatum, midbrain, cerebellum) in aged rats, accompanied by a decrease in the GSH/glutathione disulfide (GSSG) ratio, indicating diminished antioxidant capacity [87]. Similarly, MT, which is important for metal chelation and neuroprotection, shows diminished expression in aging models [88, 89]. This decline is partly attributable to reduced nuclear factor erythroid2-related factor 2 (Nrf2)-mediated transcription of GCL and MTs [90, 91]. The plasma activity of antioxidant enzymes, including glutathione peroxidase (GPX) and SOD, is considerably lower in elderly individuals than in adults [92]. In elderly individuals, blood levels of SOD1, catalase (CAT), and GPX activity are reduced and negatively correlated with age [93]. The endogenous peptide glycyl-L-histidyl-L-lysine (GHK), originally isolated from human serum, chelates Cu (II) with high affinity to form GHK-Cu. Plasma GHK levels decline markedly by age 60 [94]. Therefore, a decrease in endogenous chelating proteins such as GHK could increase free copper levels, contributing to oxidative stress damage. Furthermore, a study indicates that in the aged cardiomyocytes, chromobox 7 (CBX7) undergoes liquid-liquid phase separation with ATP7A. This interaction traps ATP7A intracellularly, impairs its membrane trafficking, and reduces copper efflux, thereby triggering cuproptosis. This mechanism reflects a defect in ATP7A's function and localization [52]. Consequently, aging progressively impairs cellular buffering systems by reducing copper-chelating proteins, antioxidants, and metal ion transport. This decline promotes free copper accumulation and copper-induced oxidative stress, which can culminate in cuproptosis and ultimately contribute to age-related cellular dysfunction.

5. Copper homeostasis imbalance and cuproptosis accelerate aging process

5.1 Telomere attrition

Telomeres are particularly vulnerable to age-related degeneration, with a small fraction of telomeric DNA being lost during each cell division because of the end replication issue when telomerase is absent, which causes gradual shortening of telomeres as age advances, and once they reach very short lengths, cells shut down their replication machinery [95]. A cross-sectional study suggests an association between dietary copper intake and leukocyte telomere length, proposing that copper may protect telomeric DNA against free radical–induced shortening by boosting antioxidant defense via SOD1 activity. However, this observational design cannot establish causality, nor does it confirm that copper intake directly elongates telomeres. Furthermore, the absence of direct measurements of oxidative stress markers or SOD activity limits mechanistic interpretation of these findings [96]. In contrast to potential protective roles, excessive metal accumulation within the body has been repeatedly associated with accelerated telomere attrition, mainly due to oxidative stress. Telomeres exhibit a high degree of sensitivity to oxidative damage [97, 98], and elevated copper levels may promote such damage through Fenton-type reactions. Higher copper exposure may lead to shortening of telomeres in leukocyte [99]. In cancer cells,

telomerase activity or telomere stress can upregulate the FDX1-thiofuranylation axis, amplifying cuproptosis [100]. This suggests a complex interplay between telomere biology and cuproptosis. Copper overload and cuproptosis may cause telomere shortening through oxidative stress mechanisms. Research indicates telomere shortening and lower telomerase activity in microglia from aged rats, but not in astrocytes. Moreover, glial telomeres in the post-mortem tissue of AD patients were markedly shorter than those in non-demented cases, suggesting inflammation may be a triggering condition [101, 102]. As microglia exhibit elevated copper levels under inflammatory conditions [77], whether copper may accelerate brain aging by promoting microglial telomere shortening via oxidative stress warrants further experimental verification.

5.2 Mitochondrial dysfunction

Reduced mitochondrial function is now understood to yield divergent effects on lifespan. In some contexts, it can increase lifespan via induction of oxidative stress resistance, mitochondrial hydrogen peroxide (H_2O_2) signalling, and xenobiotic detoxification genes [103]. This observation was confirmed in neurons of *Drosophila* and mouse, where reduced activity of electron transport chain components could prolong lifespan [104]. Current evidence suggests that cuproptosis primarily targets mitochondrial respiratory chain complexes. Cells highly dependent on mitochondrial respiration exhibit heightened sensitivity to cuproptosis, despite copper's essential role in respiratory function. Disrupted extranuclear copper utilization consequently reduces O_2 consumption capacity [105]. In this regard, we speculate that the mitochondrial respiratory electron transport chain may reduce its activity to combat cuproptosis and prevent the aggregation of lipoylated proteins which could trigger proteotoxic stress. In addition, excess copper inhibits mitochondrial respiratory chain complex IV. The copper chaperone COX17 can reactivate this complex and improve respiratory function by mediating mitochondrial copper export [106]. The long noncoding RNA (lncRNA) *CR43306* has been identified as a novel lncRNA associated with aging. In *Drosophila* models, loss of *lncRNA:CR43306* leads to partial male infertility and accelerates testicular aging, as indicated by elevated β-galactosidase activity. Further mechanistic studies demonstrated that under *lncRNA:CR43306*-deficient conditions, copper overload aggravates testicular senescence by inducing cuproptosis and cooperatively activating ferroptosis. These findings suggest copper overload triggers mitochondrial cuproptosis via the TCA cycle and engages in crosstalk with ferroptosis, collectively contributing to testicular aging [107]. Mitochondrial copper overload can partially promote renal aging and fibrosis by inhibiting mitochondrial pyruvate dehydrogenase activity [108]. Mitochondrial quality control mechanisms may play a role in inhibiting cuproptosis. Mitochondrial dynamics are responsive to copper levels: low copper concentrations induce gradual mitochondrial fusion into giant organelles, providing a compensatory mechanism for impaired oxidative phosphorylation. Conversely, higher copper concentrations downregulate fusion proteins and upregulate the fission mediator dynamin-related protein 1 (DRP1), promoting mitochondrial fission [109]. In addition, mitochondrial autophagy is involved in maintaining copper homeostasis when copper concentrations exceed physiological levels [110]. Elevated copper can trigger mitophagy through multiple pathways: Mitochondrial ROS generation, disruption of membrane potential, or Mammalian target of rapamycin (mTOR) pathway suppression [57, 111]. However, elevated copper concentrations can impair mitophagy by degrading PINK1/Parkin or suppressing the microtubule-associated protein 1A/1B-light chain 3 (MAP1LC3) expression, thereby blocking autophagosomal substrate recruitment and ultimately triggering apoptosis or pyroptosis [112, 113]. Notably, both PINK1 and Parkin critically regulate mitophagy and facilitate the removal of amyloid-β (Aβ) aggregates within neurons of the AD brain, and high copper overload impairs autophagy, thereby hindering the clearance of Aβ [114]. The exact mechanisms regulating these processes remain to be fully elucidated.

5.3 Oxidative stress

ROS are primarily generated through the mitochondrial electron transport chain where electron leakage at complexes I and III leads to superoxide formation, as well as via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and peroxisomal metabolism [115]. Under physiological conditions, the levels of ROS are strictly controlled by internal antioxidant systems such as superoxide dismutase isoforms, GPX, and GSH. However, during aging, mitochondrial dysfunction elevates ROS production while antioxidant capacity declines. These changes collectively trigger a recurring cycle of ROS accumulation and oxidative damage, reinforcing age-related cellular decline [116]. Copper is present in two states: the oxidized Cu(II)/Cu^{2+} and the reduced Cu(I)/Cu^{+}, and both participate in redox reactions [16]. The transition between Cu^{+} and Cu^{2+} states aids in electron transfer, resulting in the generation of ROS via Fenton reactions [117]. Copper overload triggers a burst of mitochondrial and NOX-derived ROS, leading to an increase in lipid peroxidation and DNA oxidation

products; this redox shift is sufficient to drive cellular senescence in liver, vasculature, brain and cartilage, providing a unifying mechanism by which copper contributes to multiple age-related diseases. Research has confirmed that elevated copper levels can promote DNA damage, specifically strand breaks and base oxidation, through the generation of free radicals [118]. Liver copper activity increases with advancing age, whilst protective hepatic enzymes such as ALDH1A1 and GSH levels decline in parallel, this copper excess generates ROS via the Fenton reaction, leading to lipid peroxidation and accumulation of the DNA oxidative damage marker 8-hydroxy-2'-deoxyguanosine (8-OHdG), thereby driving hepatic cellular senescence [68]. Integrating transcriptomics and behavior tests indicate that copper exposure shortens nematode lifespan and increases levels of ROS, the lipid peroxidation product malondialdehyde (MDA), and H_2O_2 [119].

5.4 Proteostasis imbalance, impaired autophagy clearance

Proteostasis, governed by synthesis, folding, and degradation, is essential for preventing protein misfolding and aggregation. In eukaryotic cells, key proteostatic processes, including autophagy, the ubiquitin-proteasome system, and lysosomal degradation, function to scavenge ROS-damaged proteins, clear neurotoxic aggregates, and support synaptic plasticity [120]. Dysregulation of these mechanisms underlies protein aggregation in age-related neurodegenerative diseases. Proteostasis imbalance results in the accumulation of misfolded proteins (e.g., Aβ, α-synuclein) within neural stem cells (NSCs). This proteotoxic buildup induces lysosomal dysfunction, thereby impairing the cellular clearance capacity of NSCs and suppressing their activation and proliferation. Research indicates that senescent cells exhibit copper accumulation, which is associated with alterations in copper transporters and impaired autophagy function. In senescent cells, impaired autophagic lysosomal function initiates the copper accumulation. This leads to copper being trapped within vesicles that cannot be cleared. Although the copper export protein ATP7A function normally, the high intracellular copper concentration triggers a compensatory misregulation: the cell mistakenly perceives a “copper deficiency”, thereby upregulating the expression of the copper import protein CTR1. This results in increased copper uptake into the cell. Ultimately, this combined with the original export barrier exacerbates copper accumulation within senescent cells [57]. Simultaneously, aging can weaken the cell division barrier, causing misfolded proteins to spread and accumulate in daughter cells. The combined effects of impaired clearance and aberrant protein propagation accelerate NSC functional decline and senescence phenotypes, while also directly exacerbating the pathogenesis of neurodegenerative diseases [121]. Excessive copper and its chelates can directly inhibit proteasome activity, disrupt E1/E2 ubiquitin activation and transmission, leading to interruption of ubiquitination, misfolded protein aggregation, and causing neurodegeneration [122]. In addition, Cu^{2+} can target the regions of ubiquitin that are prone to aggregation and trigger its self-oligomerization [123, 124]. Autophagy, an evolutionarily conserved degradative pathway regulated by autophagy-related genes (ATG), provides a critical safeguard. Macroautophagy, its major form, involves ATG-dependent formation of autophagosomes that encapsulate aberrant proteins, damaged organelles, and intracellular pathogens, delivering them to lysosomes for degradation [125, 126], and previously mentioned mitochondrial autophagy. Copper overload upregulates MAP1LC3, essential for autophagosome membrane formation, and promotes nuclear translocation of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, thereby enhancing autophagosome and autolysosome formation [127, 128]. mTOR is a key regulatory factor in autophagy, moderate inhibition of the mTOR pathway is thought to delay aging and prolong lifespan. However, excessive activation of the mTOR pathway may lead to cellular metabolic dysfunction and accelerated cellular aging [129]. Copper can initiate an autophagic response by affecting mTOR activity, and when mTOR is inhibited, autophagy is triggered [130]. Although copper-induced autophagy may initially be cytoprotective, exceeding a certain threshold may trigger cell death; whether this involves cuproptosis remains an open question. With aging, autophagosome biogenesis declines markedly in the brain. Age-related lysosomal dysfunction further impairs the clearance of pathogenic protein aggregates such as Aβ, leading to their accumulation and disrupting autophagic flux [131]. This establishes a vicious cycle of proteostatic failure. Notably, these aggregates can accumulate copper [132], while autophagy deficiency concurrently promotes copper accumulation. Together, these effects amplify metal-mediated neurotoxicity and accelerate disease progression. In multiple age-related neurodegenerative diseases, copper dysregulation directly exacerbates proteostasis imbalance by promoting the misfolding and aggregation of key pathogenic proteins. In AD, copper facilitates Aβ aggregation and tau hyperphosphorylation, impairing clearance mechanisms and promoting neurotoxic oligomer formation. In PD, copper accelerates α-synuclein protofibrillation. In HD, copper enhances mutant huntingtin aggregation and amyloid deposition [133]. A common consequence across these disorders is copper-mediated proteostatic

disruption, which converges on impaired protein degradation, exacerbated oxidative damage, and progressive loss of neuronal function.

5.5 Epigenetic alterations

Epigenetic alterations constitute one of the core mechanisms of aging, including DNA methylation, histone modification, chromatin remodeling, all of which play a role in controlling the aging process and contribute to age-related diseases [134]. Metal homeostasis imbalances, particularly the accumulation of copper ions, may represent a key factor exacerbating this process. S-adenosylhomocysteine hydrolase (SAHH) is a key enzyme in the methionine cycle, responsible for hydrolysing the reaction product S-adenosylhomocysteine (SAH). This step is crucial for sustaining the ongoing methylation reaction. Copper binds non-competitively to SAHH, thereby inactivating the enzyme [135]. When SAHH is inhibited, its substrate SAH accumulates intracellularly; SAH acts as a potent inhibitor of nearly all methyltransferases. Mouse models of hepatic copper accumulation exhibit transcriptional downregulation of the SAHH gene [136]. Collectively, these findings suggest that copper overload may disrupt cellular methylation capacity through SAHH inhibition, thereby exerting broad epigenetic influence on gene expression patterns. Higher circulating copper concentrations in older adults demonstrated significant positive associations with DNA methylation age acceleration metrics (PhenoAge-Accel, GrimAge-Accel, DNAm-MS), indicating accelerated epigenetic aging [137]. This association indicates that the toxicity of copper overload may accelerate epigenetic aging to exacerbate cellular fragility. With aging, the intracellular nicotinamide adenine dinucleotide (NAD) consumption increases [138], this shift inhibits the activity of sirtuin 1 (SIRT1), a deacetylase enzyme dependent on NAD^+, reduced SIRT1 activity further impacts its downstream target peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), a key regulator of mitochondrial biogenesis and function [139, 140]. The suppression of PGC-1α activity can lead to decreased transcriptional levels of the mitochondrial copper-associated cytochrome c oxidase subunits COX1 and COX2, thereby impairing mitochondrial copper utilisation and respiratory chain function [141]. Furthermore, during aging, oxidative stress impairs the function of the translocon subunit SEC61 within the endoplasmic reticulum-Golgi apparatus pathway. This prevents the ATP7A protein from being correctly targeted to the cell membrane, thereby diminishing the cell's capacity to efflux copper ions [142]. The resulting copper accumulation within cells exacerbates NAD^+ consumption by inducing cuproptosis. One hallmark of cuproptosis is the loss of Fe-S clusters, wherein NAD^+ activates the deacetylase SIRT2 to enhance NADPH production. NADPH donates hydrogen and electrons for the biosynthesis of Fe-S clusters. Consequently, elevating NADPH levels mitigates cuproptosis. However, NAD^+ consumption reduces NADPH, exacerbating Fe-S cluster protein loss and thereby inducing cuproptosis [143]. In summary, copper accumulation directly disrupts epigenetics by suppressing SAHH, forming a self-perpetuating vicious cycle alongside NAD^+ depletion, impaired mitochondrial function, and impaired copper efflux associated with aging. This ultimately leads to cellular damage via cuproptosis, thereby accelerating the aging process.

5.6 Chronic inflammation

Inflammation is now established as a key endogenous factor in aging. The senescence-associated secretory phenotype (SASP) produced by senescent cells not only promotes chronic inflammation but also induces senescence in neighbouring cells. With advancing age, the immune system experiences a decline in function, a phenomenon termed “immunosenescence”. This is characterised by reduced proliferation of immature B and T cells, diminished B and T cell receptor repertoires, and a pro-inflammatory, age-associated secretome [144]. The persistent low-grade inflammation in immunosenescence impairs copper homeostasis and alters copper transporter expression, thereby ultimately leading to copper accumulation [145]. Cuproptosis activates the cyclic GMP-AMP synthase (cGAS)- stimulator of interferon genes (STING) immune pathway. Cuproptosis-induced lipoylated protein aggregation leads to mitochondrial dysfunction and releases mitochondrial DNA (mtDNA) fragments into the cytosol, catalysing the production of cyclic GMP-AMP (cGAMP), boosting the expression of phosphorylated-interferon regulatory factor 3 (IRF3) and phosphorylated-STING proteins, subsequently, the production of type I interferon (IFN) and expression of IFN-β are increased, leading to immune and inflammatory responses in neighbouring cells [146]. Persistent activation of the cGAS-STING signalling pathway can itself drive cellular senescence [147]. Cuproptosis provides a plausible rationale for the chronic low-grade inflammation associated with aging. In aged muscle tissue, the expression of multiple cuproptosis-related genes is significantly altered, including *PDHA1*, *PDHB*, *DLAT*, *DLST*, *DLD*, *FDX1*, and *LIAS*. The expression of these genes correlates markedly with the level of immune cell infiltration, particularly with increased infiltration of dendritic cells and mast cells in aged muscle, alongside a reduction in regulatory T cells (Tregs). This suggests that cuproptosis may promote the aging process by

exacerbating inflammatory responses through its influence on the immune microenvironment [148]. In skin aging, Tregs may undergo compensatory expansion in the short term due to inflammatory accumulation, balancing excessive inflammation through immunosuppression to protect the skin from acute damage. However, prolonged natural aging or repeated photoaging activation leads to immune senescence and tissue degeneration. Acting synergistically with other immunosuppressive cells, they amplify immunosuppressive effects via IL-10, TGF-β, and ROS molecules, while promoting the persistent accumulation of senescent cells [149].

Significantly, macrophages, critical for clearing senescent cells, are themselves affected by aging and the SASP-rich environment. These aging macrophages secrete elevated levels of pro-inflammatory SASP factors like TNF-α, IL-6, and IL-1β, further reinforcing the inflammatory milieu. In a paradoxical decline, this state suppresses their own phagocytic capacity, compromising the clearance of senescent cells and creating a self-sustaining vicious cycle of inflammation and immune dysfunction that accelerates aging [150, 151]. Copper-triggered phosphorylation of p38 mitogen-activated protein kinase (MAPK) in microglia caused a transition to the M1 phenotype and foam cell formation, promoting inflammation [152]. Furthermore, copper could increase vulnerability to inflammatory triggers by inducing apoptosis in macrophages or neutrophils, leading to reduced phagocytic functions and unsustainable immune responses [153]. Copper overload triggers a distinctive form of mitochondrial stress, resulting in ATP depletion and activation of AMP-activated protein kinase (AMPK). The activated AMPK exerts a dual function: it directly promotes the execution of cuproptosis while concurrently phosphorylating the nuclear protein high-mobility group box 1 (HMGB1), facilitating its active release into the extracellular space. HMGB1 acts as a damage-associated molecular pattern (DAMP) that binds specifically to the advanced glycosylation end product-specific receptor (AGER), leading to macrophage activation and a robust inflammatory response, and this cascade ultimately contributes to the initiation and progression of inflammatory diseases [154].

Aging deteriorates the epigenetic silencing of human endogenous retroviruses (HERVs), primarily through declining DNA methylation and histone deacetylation. This permits HERV reactivation and the generation of transmissible retrovirus-like particles (RVLPs). These particles can induce senescence in neighboring healthy cells and promote a state of systemic, low-grade inflammation, partly through activation of the cGAS-STING pathway. Notably, age-related HERV derepression is further amplified by exogenous viral infections. In the context of immunosenescence, pathogens such as SARS-CoV-2 or Epstein-Barr virus (EBV) can act as potent catalysts for HERV expression. The resulting HERV-derived double-stranded RNA and DNA are recognized by innate immune sensors, including cGAS-STING and Melanoma differentiation-associated protein 5/Mitochondrial antiviral-signaling protein (MDA5/MAVS), driving a sustained production of type I interferons and pro-inflammatory cytokines [155]. This feed-forward loop exacerbates the chronic inflammatory state characteristic of inflammaging, thereby linking exogenous viral challenges to the acceleration of age-related inflammation. Moreover, viral infections themselves are associated with cuproptosis [13]. The cuproptosis pathway may be associated with the host response to severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) infection and disease severity. Furthermore, SARS-CoV-2 reduces GPX4 expression, thereby promoting ferroptosis, and it also drives PANoptosis (an RCD pathway integrating pyroptosis, apoptosis, and necroptosis) via the Janus kinase/signal transducer and activator of transcription 1 (JAK/STAT1) axis [156]. The hepatitis B virus (HBV) HBx protein exhibits sequence similarity to the host copper-metabolism protein copper metabolism MURR1 domain-containing 8 protein (COMMD8). Furthermore, bioinformatic analyses suggest that lncRNAs correlated with cuproptosis genes are dysregulated in HBV-induced hepatocellular carcinoma, however, it is still speculative whether HBV directly affects cuproptosis [157, 158]. Moreover, cuproptosis itself exhibits crosstalk with other forms of RCD. Ferroptosis is not only a crucial mechanism of cell death during aging but also a key bridge linking iron metabolism imbalance, oxidative stress, inflammatory responses, and age-related diseases [159]. Ferroptosis and cuproptosis exhibit close interactions across multiple pathways, including mitochondrial metabolism, GSH metabolism, and autophagy regulation, which collectively drive inflammatory cell death [160]. PANoptosis is a recently identified form of PCD that integrates aspects of pyroptosis, apoptosis, and necroptosis. PANoptosis releases inflammatory factors DAMPs, inducing cell death and further exacerbating cellular aging [161]. *In vitro* cultured tumor cells and in vivo mouse tumor models, cuproptosis induces mitochondrial proteotoxic stress and respiratory inhibition, leading to the release of mtDNA into the cytoplasm [162]. Z-DNA-binding protein 1 (ZBP1) is a cytosolic nucleic acid sensor that recognizes viral Z-form nucleic acids and self-nucleic acids (such as mtDNA) through its Zα domain, inducing inflammatory cell death [163, 164]. Exposure to copper nanoparticles activates ZBP1 and upregulates key proteins mediating apoptosis, pyroptosis, and necroptosis, thereby inducing PANoptosis [165, 166]. Overexpression

of the cuproptosis core gene *FDX1* significantly enhances the antitumor effect of the copper ionophore ES in diffuse large B-cell lymphoma, potently sensitizing cells to ES-induced IFN-β-dependent PANoptosis both *in vitro* and *in vivo* [167]. However, there is currently a lack of direct evidence to demonstrate whether cuproptosis is sufficient to independently trigger PANoptosis, and the precise molecular links and temporal regulation between these two processes await further clarification.

Gut dysbiosis is common in WD, and copper exposure itself alters gut microbiota such as enriching proinflammatory taxa, reducing Firmicutes/Bacteroidetes ratio, and compromises gastrointestinal barrier integrity through inflammation and oxidative stress [168, 169]. This creates a self-reinforcing loop: copper-induced gut damage exacerbates inflammation, which further disrupts copper homeostasis. Copper-induced inflammation and oxidative stress reduce the expression of tight junction proteins and promote their degradation [170, 171]. This may impair mucosal permeability and enable translocation of gut microbiota-derived LPS and toxins into the bloodstream, thereby triggering peripheral inflammation. Although BBB disruption has been documented in aging mice, significant cognitive impairment emerges only following an inflammatory challenge. Under healthy aging, the BBB undergoes gradual damage, encompassing oxidative stress, epigenetic changes, genomic instability, telomere attrition and dysregulation of cell signalling, with no significant disease manifestations. However, the disruption accelerates markedly upon the onset of an inflammatory response, causing significant cognitive decline [172, 173]. Copper overload significantly increases BBB permeability through oxidative stress, HMGB1-mediated inflammation, and downregulation of tight junction proteins claudin-1, claudin-3, and claudin-5, impairing the BBB [174, 175]. The gut-brain axis, a bidirectional communication network connecting the gastrointestinal tract with the CNS, involves intricate interactions mediated by gut microbiota, hormones, the enteric nervous system, and nutrients. Within this axis, the gut microbiota serves as a primary regulator of neurological and behavioral functions. Moreover, the gut microbiome of individuals with AD reportedly exhibits taxonomic shifts across multiple levels, featuring reduced *Firmicutes* abundance, increased *Bacteroidetes*, and a decline in *Bifidobacterium* [176, 177]. In summary, copper-induced intestinal barrier disruption acts as a critical initiating event in neurodegenerative progression, disrupting gut homeostasis, triggering systemic inflammation, and amplifying CNS neurotoxicity to contribute to AD, PD, and other age-related neurodegenerative diseases. In brain aging, aged neurons exhibit decelerated protein synthesis, decreased neurotrophin expression, synaptic loss, decreased neuroplasticity and an elevated inflammation state compared to adult brains [178-182]. These neuronal alterations contribute to age-related cognitive and behavioural decline. Emerging research indicates that elderly patients often exhibit elevated serum copper levels [183, 184]. Increased Cu^{2+} content is associated with aging, and sublethal Cu^{2+} exposure induces premature cellular senescence [185]. On the other hand, neuroimmune responses associated with glial cells are vital for maintaining synapses [186]. Activated microglia secrete pro-inflammatory factors; elevated copper levels have been associated with increased ATP7A expression in microglia in response to the pro-inflammatory cytokine interferon-γ. Interferon-γ triggers profound copper dysregulation through three coordinated mechanisms: copper-dependent relocalization of ATP7A from Golgi to post-Golgi vesicles, enhanced cellular copper influx, and upregulation of CTR1 expression [187]. These findings indicate that neurodegeneration-associated neuro-inflammation drives microglial copper transport dysregulation, which may underlie changes in copper homeostasis in AD. This bidirectional relationship may be mutually causative. Microglial copper sequestration may also represent an endogenous neuroprotective response in AD pathogenesis. Various forms of cell death significantly influence the inflammatory environment surrounding neurons in the aging brain, resulting in neuronal dysfunction to neuronal loss. Pro-inflammatory DAMPs released from lysed neurons exacerbate neuronal dysfunction by stimulating an inflammatory response in glial cells [188]. This creates a vicious cycle.

6. Cuproptosis in age-related diseases

6.1 Age-related neurodegenerative diseases

Age-related neurodegenerative diseases have a severe impact on the elderly, stripping them of their memories, altering their social interactions, and ultimately removing their independence. Typical neurodegenerative diseases, such as AD, PD, and HD, almost all were observed to have changes in copper levels and exhibits region-specific dysregulation [189-191].

6.1.1 Alzheimer's disease (AD)

Aging represents the most critical risk factor for AD, with advancing age, the brain undergoes systemic and specific physiological alterations that markedly increase susceptibility to the onset of AD. Molecular hallmarks accumulated during aging, including cellular senescence, chronic neuroinflammation, mitochondrial dysfunction, oxidative stress, and disrupted protein homeostasis, are closely linked to AD pathologies such as Aβ deposition,

abnormal tau acetylation and hyperphosphorylation, and neurofibrillary tangle formation. Tau pathology often precedes or occurs independently of Aβ during aging, serving as a pivotal link between aging and cognitive decline. Through its interplay with aging mechanisms via signalling pathways such as glycogen synthase kinase-3 (GSK-3), cyclin-dependent kinase 5 (CDK5), mTOR, and SIRT1, tau pathology emerges alongside Aβ as a critical intervention target [192]. Copper homeostasis imbalance plays a multifaceted role in AD pathogenesis by promoting both Aβ and Tau pathology [193]. Cu^{2+} binds to Aβ via specific histidine and tyrosine residues, facilitating aggregation and generating oxidative stress through Fenton reactions. Concurrently, copper induces Tau hyperphosphorylation and neurofibrillary tangle formation, impairing proteostasis and microtubule stability [194, 195]. Amyloid precursor protein (APP) participates in copper efflux, its aggregation affects intracellular copper availability and SOD1 activity [196, 197]. Age-related decline in copper chaperones further exacerbates oxidative damage [198]. Collectively, copper-mediated mechanisms converge to disrupt protein clearance, promote toxic oligomerization, and accelerate neuronal dysfunction in AD [199]. The cuproptosis-related gene ATP7B is mainly involved in copper excretion, and mutations in ATP7B disrupt copper excretion, increasing the risk of AD by causing elevated serum non-copper-binding Cu levels [200]. LIAS is the primary enzyme involved in the biosynthesis of lipoic acid, and its downstream protein α-lipoic acid (ALA) is an important cofactor for mitochondrial respiratory enzymes. In AD cell models, ALA significantly improves mitochondrial respiratory function and reduces intracellular ROS levels [201]. This suggests that ALA may enhance the resistance of neurons to cuproptosis. In the AD neuronal model, the expression level of FDX1 was significantly increased, and knockdown of *FDX1* expression reduced the lipidation levels of *DLAT* and *DLST* in neurons, and mitigated cuproptosis [51]. Microglia-derived extracellular vesicles mediate the delivery of pyruvate kinase M2 (PKM2) to hippocampal neurons. This process upregulates DLAT protein, induces cuproptosis, and exacerbates cognitive deficits in AD mouse model [202].

6.1.2 Parkinson's disease (PD)

PD pathogenesis may originate from gut dysbiosis, which triggers α-synuclein aggregation. These aggregates propagate in a prion-like manner along the vagus nerve to the olfactory bulb, spinal cord, and enteric nervous system, ultimately accumulating in the substantia nigra pars compacta. Additionally, compromised BBB permeability provides an alternative pathway for α-synuclein spread to the substantia nigra [203-205]. Elevation of iron levels has been documented in the brains of PD patients [206], potentially attributable to dysregulated iron metabolism and diminished copper levels. Excess copper in the medulla oblongata of PD mouse models promotes α-synuclein aggregation, while copper reduction mitigates this effect [207]. Cuproptosis-related genes contribute to PD risk: Bioinformatics implicates *KIAA0319* (neuronal migration/adhesion), angiotensin II receptor type 1 (*AGTR1*), and solute carrier family 18 member A2 (*SLC18A2*) (vesicular monoamine transporter) in PD pathogenesis [208]. Besides, PD may involve synaptic dysfunction linked to ATP7B, NFE2L2, and metal-regulating transcription factor MTF1 [209]. Moreover, DLD and FDX1 overexpression reportedly enhances immune cells proliferation and migration, implicating these genes in PD pathogenesis [210].

6.1.3 Huntington's disease (HD)

HD, an autosomal dominant neurodegenerative condition, is identified by involuntary choreiform movements, progressive deterioration in speech, mobility, cognition, and swallowing function [211]. It is caused by CAG trinucleotide repeat expansions in exon 1 of the huntingtin (*HTT*) gene, resulting in mutant HTT protein with an elongated polyglutamine (polyQ) tract. This mutation promotes protein aggregation and confers neurotoxicity [212, 213]. Clinically, elevated copper levels are detected in the CSF of HD patients early in the disease course, preceding other biomarker changes, though not reflected in plasma [191]. Copper overload promotes mutant HTT aggregation and enhances the accumulation of thioflavin S-positive β-amyloid structures in Htt aggregates. Copper also influences HD pathology by regulating HTT exon1-polyQ accumulation through direct binding to HTT exon1 [214]. Therapeutic strategies targeting copper homeostasis show promise: either by regulating copper transporter expression or by using copper chelators such as BCS or clioquinol (CQ), both of which ameliorate HD phenotypes in *Drosophila* models [215].

6.2 Hypertension

Hypertension, as a major risk factor for cardiovascular diseases (CVDs), and is further recognised as a pathological state that accelerates vascular aging. Vascular aging also serves as a pathological driver of CVDs and cerebrovascular diseases. Its core mechanism involves degenerative changes in the arterial tunica layer, resulting in vascular lumen enlargement, endothelial dysfunction, elastin fragmentation, and disorganized smooth muscle cell arrangement. These alterations impair tissue perfusion, thereby compromising oxygenation,

nutrient delivery, and waste removal, which subsequently lead to multi-organ dysfunction [216-218]. This increases susceptibility to hypertension [219]. Exposure of human diploid fibroblasts to subcytotoxic concentrations of copper results in premature senescence [57]. The copper-dependent Lysyl oxidase (LOX) family maintains vascular wall integrity through covalent cross-linking of elastic fibers into insoluble matrices [220]. Elevated copper levels enhance LOX-mediated extracellular matrix cross-linking, contributing to arterial stiffness and serving as a crucial structural basis for the development of hypertension [221]. Moreover, copper activates the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, driving neointimal hyperplasia and pathological vascular remodeling in injured vessels [222], thereby accelerating vascular aging. Furthermore, copper exacerbated age-related vascular repair dysfunction via elevated thrombospondin-1 (TSP-1), which inhibits endothelial progenitor cell function in mice, and influences tissue repair and regeneration [223, 224]. This repair mechanism creates a vicious cycle with age-related vascular functional decline. Cuproptosis not only accelerates vascular calcification by inducing mitochondrial dysfunction and protein toxicity stress, but also directly contributes to hypertensive cardiac remodelling. Compared with non-calcified aortas, calcified aortas exhibited accumulation of FDX1, a key mediator of cuproptosis, alongside elevated copper ion levels, elevated copper ion concentrations lead to downregulation of ATP7A expression and increased heat shock protein 70 (HSP70) levels, inducing cuproptosis. This subsequently results in myocardial fibrosis, mitochondrial damage, and cardiac dysfunction. Mitochondrial dysfunction promotes vascular calcification by affecting the activity of calcification-regulating proteins, thereby inducing hypertension through the promotion of inflammation and cellular senescence. SIRT7 and elabela (a recently identified peptide) alleviate hypertension-related damage by modulating the cuproptosis signalling pathway [225, 226].

6.3 Atherosclerosis

Atherosclerosis is a chronic inflammatory disorder defined by the progressive deposition of plaques on arterial walls, which eventually results in arterial narrowing and stiffening, driving disease progression [227]. Aging represents a critical risk factor for atherosclerosis, as age-related changes in immune cells significantly impact disease pathogenesis, during this process, T-cell populations not only undergo a quantitative decline but also accumulate intracellular cholesterol [228]. As key immune regulators in atherosclerosis, Tregs play a protective role by restraining excessive inflammatory responses, however, this function is compromised during aging. Tregs exhibit more pronounced senescence compared to conventional T cells, which impairs their ability to inhibit atherosclerotic progression [229]. Recent research has found a strong link between elevated serum copper concentrations and atherosclerotic plaque development [230]. The intima of vessels with atherosclerotic lesions has higher Cu levels compared to healthy vessels [231]. In the aforementioned hypertension, excessive copper may exacerbate vascular age-related pathological alterations and amplify oxidative stress and local inflammatory responses, thereby accelerating the progression of atherosclerosis. Elevated copper levels can also promote the formation of oxidized low-density lipoprotein (ox-LDL) from LDL particles, thereby driving the development of atherosclerosis [232, 233]. Moreover, an oxygen-deficient or glycolytically active inflammatory microenvironment has been shown to suppress cuproptosis. From a metabolic perspective, Tregs are dependent on oxidative phosphorylation rather than glycolysis; hyperactivation of the glycolytic pathway further inhibits Treg cell function [234], and this metabolic profile renders Tregs more susceptible to cuproptosis than other T-cell subsets. The cuproptosis-associated gene *LIAS* promotes Treg generation and enhances their immunosuppressive capacity by maintaining mitochondrial homeostasis and mitigating oxidative stress [235]. Experimental validation in atherosclerotic mice confirms this effect, *LIAS* overexpression significantly increases Treg numbers while reducing T-cell infiltration into arterial lesions [53], highlighting the close relationship between cuproptosis and Tregs. Further experimental evidence from atherosclerotic model mice reveals a detrimental phenotypic shift in Tregs: these cells not only lose their core function of suppressing T-cell proliferation but also acquire the ability to promote B-cell activation via interleukin-21 (IL-21), directly accelerates atherosclerotic progression [228]. Collectively, these findings indicate that increased copper levels contribute to the pathogenesis of atherosclerosis, likely by modulating cuproptosis and Treg function.

6.4 Type 2 diabetes mellitus (T2DM)

T2DM is one of the age-related diseases, with nearly half of the elderly population affected by diabetes, and the treatment of diabetes continues to present challenges [236]. Copper exhibits a significant association with T2DM and its complications. Dietary copper intake may be positively related to the risk of T2DM mellitus [237]. Serum copper levels correlate positively with the risk of developing T2DM and with glycaemic control. Elevated

urinary copper levels are associated with impaired pancreatic β-cell function, suggesting that excessive copper may exert toxic effects on pancreatic β-cells, impairing their insulin-secreting capacity and thereby disrupting glucose homeostasis even in the early stages of the disease or during the prediabetic phase [238]. Serum copper levels are elevated in patients with T2DM, an abnormality potentially linked to systemic oxidative stress in the disease state. Excess copper ions can generate ROS via the Fenton reaction, thereby activating stress signalling pathways such as p38 MAPK and Jun *N*-terminal kinase/stress-activated protein kinase (JNK/SAPK). This disrupts insulin signalling and promotes insulin resistance. Concurrently, copper induces the aggregation of pancreatic amyloid peptides into amyloid fibrils, directly impairing β-cell function and reducing their mass [239]. ASH2L is a regulator of histone modification-related Histone H3 lysine 4 trimethylation (H3K4me3), triggers genetic transcription, and hyperglycaemia upregulates ASH2L in endothelial cells, which activates STEAP4 transcription by promoting H3K4me3 enrichment at its promoter. This subsequently synergises with CTR1 to enhance copper uptake, leading to endothelial dysfunction via chronic inflammation and oxidative stress [240]. Persistent hyperglycemia induces advanced glycosylation end products (AGEs), excessive AGEs and copper in diabetes upregulated ATF3/SPI1/SLC31A1 signaling, resulting in abnormal accumulation of copper ions within cardiomyocytes and cuproptosis [241].

6.5 Osteoporosis (OP)

OP is a metabolic bone disorder marked by an imbalance between bone formation by osteoblasts and bone resorption by osteoclasts, resulting in increased bone resorption and gradual bone loss, aging is a key factor in osteoporosis [242]. Aging induces lineage skewing of bone marrow mesenchymal stem cells (BMSCs), shifting their differentiation trajectory from osteogenesis toward adipogenesis; this shift directly diminishes the pool of functional osteoblasts, thereby impairing bone formation [243]. Concurrently, aged bone marrow adipocytes secrete a repertoire of SASP factors, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and matrix metalloproteinases (MMPs); these factors propagate the senescence phenotype to neighboring osteoblasts, osteoclasts, and BMSCs, promoting local accumulation of senescent cells and further exacerbating bone metabolic disorders [244]. The p53/p21 pathway is an archetypal regulator of cellular senescence that serves as a critical molecular bridge linking aging, cuproptosis, and OP. Beyond initiating cell cycle arrest to trigger senescence in bone-related cells (such as osteoblasts and osteoclasts) [245], the p53/p21 pathway also modulates key molecular events of cuproptosis: it downregulates the expression of glutamine cysteine ligase (GCL, a rate-limiting enzyme for GSH synthesis) and Fe-S cluster assembly proteins, thereby reducing intracellular GSH levels and disrupting Fe-S cluster homeostasis, increasing cuproptosis sensitivity [246, 247]. Aging reduces the expression of estrogen-related receptor α (ERRα), a transcription factor that directly regulates the expression of mitochondrial GLS, the rate-limiting enzyme in glutamine catabolism [248]. Decreased GLS activity not only diminishes the production of α-ketoglutarate (a key metabolite for osteoblast energy metabolism via the TCA) and impairs osteoblast differentiation [248]. In osteoclasts, aberrantly accumulated copper ions drive excessive bone resorption via Fenton reaction that generates substantial ROS. These ROS exert dual effects: they directly induce oxidative stress damage in osteoclasts, and more importantly, activate the nuclear factor κB (NF-κB) signaling pathway to upregulate the expression of receptor activator of NF-κB, a key receptor that binds to receptor activator of nuclear factor kappa-B ligand (RANKL) which secreted by osteoblasts to initiate osteoclast activation [249]. Additionally, ROS stimulate the generation of osteoclastogenic cytokines such as IL-1, IL-6, IL-7 in osteoclasts and surrounding immune cells [250], forming a positive feedback loop of "ROS-cytokine-osteoclast activation" that amplifies bone resorption. Beyond direct cellular effects, cuproptosis also modulates bone metabolism indirectly via extracellular vesicles (EVs), downregulating osteogenesis-promoting miRNAs and upregulating osteoclastogenesis-promoting miRNAs. When internalized by neighboring bone cells, these EV-borne miRNAs regulate the expression of downstream target genes, thereby altering the differentiation and function of recipient cells and contributing to OP progression [251, 252].

6.6 Age-related macular degeneration (AMD)

Age-related macular degeneration (AMD) is a progressive neurodegenerative condition that progressively impacts the macula, the central region of the retina essential for high-acuity vision. The clinical hallmarks of AMD, which include degeneration of macular cells and pathological neovascularization, lead to progressive blurring and distortion of central vision, as well as the development of central scotomas. Research indicates that copper levels rise with age in the brains and retinas of both humans and mice [253]. The aging retinal pigment epithelium (RPE) cells are more susceptible to oxidative stress [254], and accumulation of copper may induce oxidative stress, leading to cellular damage and dysfunction. The single-sample gene set enrichment analysis (ssGSEA) revealed

that cuproptosis was the most significantly enriched form of RCD in the AMD-affected retina, particularly within the RPE. The pathways that were up-regulated in RPE affected by high levels of cuproptosis in AMD involved cellular senescence, inflammation, and cell death, indicating that cuproptosis could be a potential factor in AMD pathogenesis: age-related copper accumulation induces cuproptosis in retinal and RPE cells, resulting in primary macular damage. The subsequent release of DAMP from these dying cells then propagates a local inflammatory response, inflicting secondary injury and ultimately driving AMD progression [255].

7. Therapeutic implication

In recent years, therapies aimed at targeting copper homeostasis, including copper supplementation and suppression of copper overload, have been carried out with promising results, albeit with some limitations. Accordingly, the main approach is to reduce cellular copper levels and decrease the production of free radicals to inhibit cuproptosis [256]. Given the role of cuproptosis in the pathological mechanisms of age-related diseases, targeting this process could offer promising therapeutic opportunities (Fig. 4).

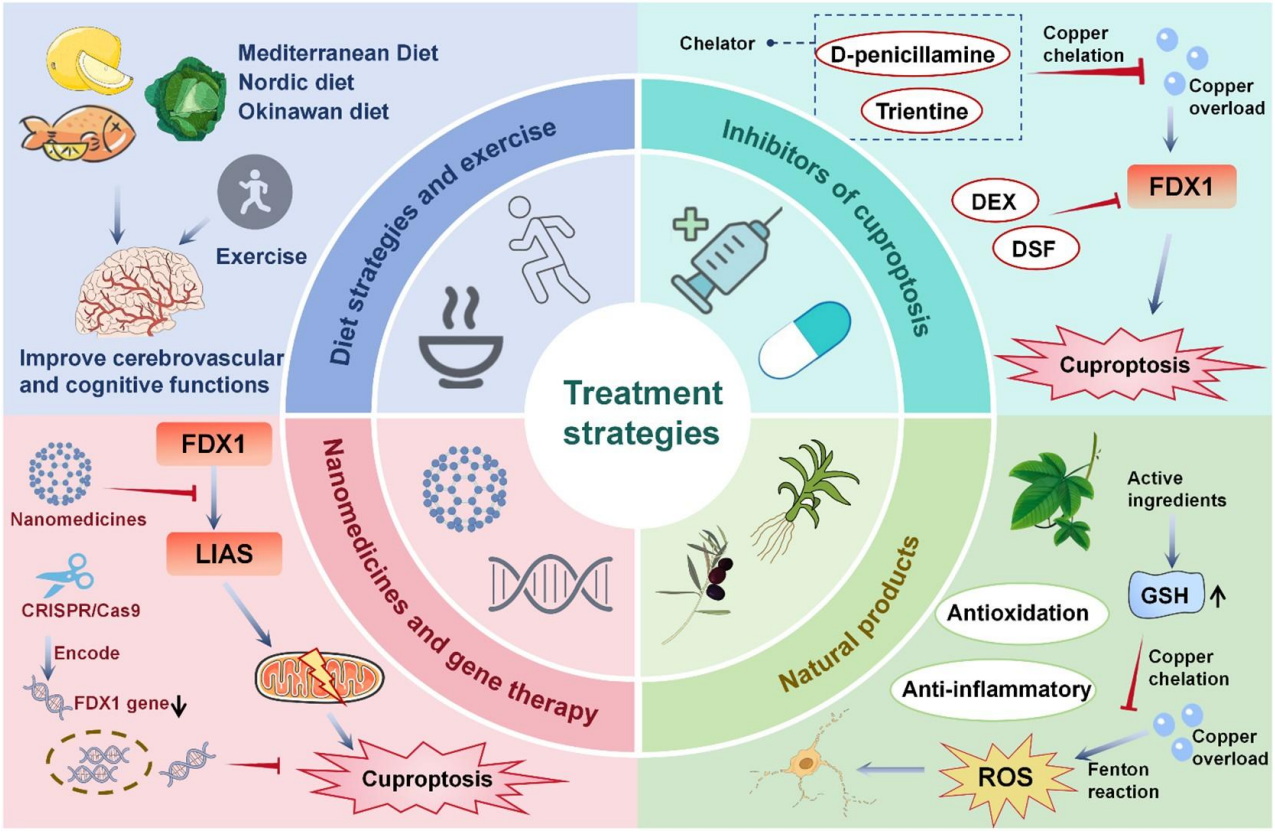

Figure 4. Therapeutic interventions by targeting cuproptosis. This figure summarizes diverse therapeutic interventions to modulate cuproptosis. Dietary patterns (e.g., Mediterranean, Nordic, and Okinawan diets) and regular physical exercise contribute to improved cerebrovascular and cognitive function, establishing a foundational protective context. Among cuproptosis inhibitors, copper chelators D-penicillamine and trientine bind to excess copper through chelation, thereby downregulating the expression of FDX1 protein to achieve the inhibition of cuproptosis. DEX and DSF also exert an inhibitory effect on cuproptosis by downregulating the FDX1 protein. A large number of active ingredients contained in natural medicines possess anti-inflammatory and antioxidant properties; they can increase the levels of copper-binding proteins like GSH and reduce the production of ROS, thus counteracting copper-induced neurotoxicity. Some nanomedicines are capable of downregulating the FDX1/LIAS pathway to inhibit cuproptosis. Furthermore, the gene editing tool CRISPR/Cas9 can be used to target the FDX1 gene, and by downregulating the expression of this gene, it can suppress the occurrence of cuproptosis.

7.1 Copper chelators: reducing copper overload

To mitigate harmful reactions of excess copper and reduce its levels, chelating agents are essential for sequestering it and attenuating the extent of cellular damage [257]. Copper chelators are currently the most important modality for treating copper overload. D-penicillamine and trientine serve as first-line oral copper chelators and form the cornerstone of the standard pharmacological management of WD, and their use is particularly

recommended in patients with significant liver disease [258]. D-Penicillamine remains a conventional therapeutic agent yet carries a substantial burden of adverse effects, including early sensitivity reactions, bone marrow suppression, and dermatological manifestations [259-261]. Of particular concern is the exacerbation of neurological symptoms, which occurs more frequently in those with pre-existing neurological involvement at treatment initiation or in those receiving concurrent DA receptor antagonists [262]. Trientine is frequently used as an alternative to D-penicillamine for patients with intolerance, offering comparable efficacy and superior tolerability. Trientine tetrahydrochloride (TETA4, Cuvrior) is an improved form of trientine, featuring a distinctive, room-temperature stable polymorphic form that addresses a key limitation of prior trientine formulations. The Phase III CHELATE trial demonstrated that in stable adult patients, TETA4 maintained copper homeostasis with non-inferiority to D-penicillamine while exhibiting superior safety. Common adverse events were limited to mild headaches and abdominal pain, with no reports of serious adverse events (ClinicalTrials.gov reference: NCT03539952). Regulatory approval for TETA4 was obtained from the US Food and Drug Administration in April 2022, with authorizations extending to the European Union, the United Kingdom, China, and other regions, for use in both adult and pediatric WD patients who are intolerant to D-penicillamine. This medication offers patients a more convenient, effective, and well-tolerated long-term treatment option [263-265]. Most immune-mediated adverse reactions induced by D-penicillamine can be managed with concomitant steroid therapy [266, 267]. Dimercaptopropane sulfonate (DMPS) and dimercaptosuccinic acid (DMSA) are two thiol-based chelators effective against a spectrum of heavy metals, including lead, mercury, cadmium, and copper. In China, both chelators are clinically employed to eliminate excess copper in WD. A randomized controlled trial demonstrated that combination therapy with DMPS and DMSA yields a more pronounced copper-elimination effect compared to D-penicillamine, accelerates the removal of metals from brain tissue, and leads to greater improvement in neurological symptoms [268, 269]. The BBB-permeable chelators PBT2 and CQ have emerged as promising candidates for rectifying cerebral metal imbalance. Their mechanism involves chelating copper from Aβ aggregates and effectively inhibiting the Fenton reaction in AD. PBT2 was clinically trialed as an AD therapy but failed to progress beyond phase II, and CQ is confined to preclinical research due to neurotoxic side effects but has shown potential in anti-aging studies, prolonging lifespan and improving motor activity in Alzheimer's flies [270-272]. Tetrathiomolybdate (TTM) possesses potent copper-chelating capabilities and relatively mild side effects, though its long-term safety and efficacy remain unclear, and is currently used in preclinical studies [273, 274]. The copper chelator ATN-224 reduces hepatic copper levels, restores ALDH1A1 activity, and reverses oxidative damage in liver tissue alongside elevated inflammatory factors IL-6 and TNF-α in aged mice [68]. Various copper chelators, by efficiently removing excess copper, represent potentially effective pharmaceuticals for curbing cuproptosis.

However, clinical copper chelators, such as D-penicillamine and trientine, exhibit broad metal-binding selectivity. In addition to reducing copper load, they readily compete for essential ions like zinc, iron, and calcium [275-277]. This limited specificity raises particular concern in aging populations, where systemic metal balance is already vulnerable. The tight metabolic coupling between copper and zinc further complicates their use. Zinc deficiency promotes immune aging and increases the incidence of age-related diseases [278]. Copper chelators may indirectly influence zinc homeostasis during copper excretion. For example, D-penicillamine therapy may initially exacerbate neurological symptoms by mobilizing labile copper pools, whereas subsequent treatment with zinc supplements often leads to clinical improvement. This observation implies that certain chelators may disrupt zinc-dependent cellular protection, possibly by redistributing intracellular copper or interfering with copper zinc interactions. Moreover, zinc supplementation itself reduces copper absorption by upregulating MT expression, further highlighting the tightly coupled regulation of copper and zinc metabolic pathways [279]. In the context of aging and systemic inflammation, serum copper levels tend to rise while zinc concentrations decline. This altered serum copper to zinc ratio is clinically significant, as an elevated serum copper to zinc ratio has been linked to increased mortality in the older population [280]. Copper chelators such as bathocuproine disulfonate and cuprizone exert an off-target effect by depleting bioavailable copper, which disrupts the copper-dependent, PrP(C)-mediated allosteric inhibition of N-methyl-d-aspartate receptor (NMDARs). This leads to increased glycine affinity, loss of receptor desensitization, and sustained Ca^{2+} influx, thereby exacerbating excitotoxic neuronal injury [281]. Therefore, although copper chelation therapy remains the primary treatment for copper-overload disorders, its lack of metal specificity may lead to deficiencies in essential ions and potentially exert non-targeted regulatory effects on copper-dependent biological pathways. Moreover, based on findings from a breast cancer model, the copper chelator TTM, while lowering serum copper, may exert broader, off-target effects on the aging microenvironment by disrupting copper-dependent processes critical for

vascular integrity and tissue repair. These effects include a reduction in circulating progenitor cells and suppression of LOX activity and collagen cross-linking, thereby impairing extracellular matrix homeostasis [282]. Furthermore, copper chelators may interact with chronic medications taken by elderly individuals, potentially leading to therapeutic interference and cumulative toxicity under polypharmacy conditions. For example, trientine should be taken two hours apart from iron supplements and zinc supplements [258].

7.2 Cuproptosis pathway inhibitors

There remains considerable scope for developing specific cuproptosis inhibitors. The antihypertensive drug ACT-132577, already approved for clinical use, has been shown to target copper homeostasis, it functions by enhancing E3 ubiquitin-protein ligase NEDD4-like (NEDD4L)-mediated ubiquitination and degradation of the copper transporter CTR1, lowering cellular copper levels, suppressing cuproptosis and ultimately alleviating pathological vascular remodeling, highlighting a novel mechanism of action for this approved compound [283].The anesthetic and sedative drug dexmedetomidine (DEX) was reported to increase cell survival by blocking the FDX1-mediated cuproptosis pathway during brain injury [284]. Disulfiram (DSF) inhibits FDX1 expression, which reduces the ability of FDX1 to convert Cu^{2+} to more toxic levels of Cu^{+} and decreases the transport of ATP7A and ATP7B. Inhibition of CTR1 affects Cu uptake, thereby inhibiting the cuproptosis pathway [285]. Chlorzoxazone decreases neuronal death in the hippocampus and reduces inflammation, thereby rescuing cognitive deficits in AD, and analysis using the Enrichr database revealed that chlorzoxazone inhibits cuproptosis by targeting lipoyltransferase 1 (LIPT1), FDX1, and DLD [286]. Artesunate targets metallothionein 2A (MT2A) in astrocytes to promote intracellular copper efflux, thereby inhibiting the FDX1-DLAT axis and improving neurological function in PD. This study provides the first evidence that an antimalarial drug can suppress cuproptosis-induced neurodegeneration [287]. Trilobatin directly targets FDX1, blocking the reduction of Cu^{2+} to Cu^{+} and inhibiting DLAT oligomerisation. This reduces the number of cuproptosis-positive myocardial cells, improves cardiac function, and suppresses myocardial fibrosis [288]. Dapagliflozin inhibits the HIF-1α/TGF-β pathway while simultaneously reducing FDX1 expression in the myocardium of mice with myocardial infarction, thereby suppressing cuproptosis and improving cardiac function and myocardial fibrosis [289]. In summary, the current exploration of cuproptosis inhibitors is still nascent. The repertoire of specific inhibitors remains limited, and their full therapeutic potential requires further extensive validation.

7.3 Improving aging microenvironment: reducing sensitivity to cuproptosis

7.3.1 Diet strategies and exercise

Although copper supplementation and copper chelation remain controversial [290], maintaining good eating habits is considered to prolong life. Traditional healthy dietary patterns, such as the Mediterranean diet, Nordic diet, Okinawan diet, and contemporary dietary patterns, such as HEI-2015, AHEI, and DASH diets, can lower the risk of age-related diseases, improve cognitive function, and are associated with lower mortality and healthier life expectancy [291]. The combined intake of dietary nutrients, including copper, was negatively associated with accelerated aging [292]. Indeed, dietary copper intake does not equate to serum and plasma copper levels, as copper levels in the blood are influenced by various factors. Thus, slightly increasing the consumption of certain dietary nutrients and maintaining a balanced diet could be crucial strategies to slow down the aging process. Exercise training is believed to reduce vascular inflammation and inhibit endothelial dysfunction, improve cerebrovascular and cognitive functions [293]. Furthermore, physical activity can effectively alleviate the adverse effects of elevated serum copper levels on aging by regulating inflammation levels [294].

7.3.2 Natural medicines

Natural products exhibiting antioxidant, anti-inflammatory, and metal ion chelating properties may constitute a promising area of research for the treatment of cuproptosis and aging. Some herbal active ingredients can increase copper-chelating proteins, such as GSH, which has the potential to inhibit cuproptosis. Potential natural anti-aging agents with the capacity to inhibit cuproptosis and reduce copper levels have been summarised in Table 2. Moreover, turmeric, a medicinal herb used for wound/bite/burn management and treating ocular/acne infections, contains bioactive curcuminoids. These compounds and their synthetic derivatives demonstrate copper toxicity antagonism [295], however, it exhibits limited ability to cross the BBB and must be encapsulated by drug carriers [296]. A complex biopolymer isolated from the flowering parts of *Agrimonia eupatoria* L., a polyphenolic glycoconjugate, acts as an effective copper reducing agent and a powerful antioxidant [297]. As principal bioactive components of Astragalus, vanillic acid and daidzein synergistically enhance SOD and CAT activities, GSH levels, and

transcriptional activation of antioxidant genes [298]. Obovatol ameliorates glia-mediated neurotoxicity and oxidative damage induced by GSH depletion [299]. Rutin attenuates Cu-triggered cerebral damage via synergistic suppression of oxidative stress and neuroinflammation [300].

Table 2. Cuproptosis targeted natural products for the treatment of aging.

Sources	Bioactive constituents	Function for anti-aging	Function for inhibiting cuproptosis	Refs.
Dracoephalum moldavica L. (TFDM)	Total flavonoid extract	Antioxidant.	Cu-Aβ↓, binding to copper ions to reduce the copper-induced cytotoxicity.	[309]
Epimedium brevicornum Maxim	Icariin	Antioxidant; Anti-inflammatory; Improve mitochondrial function; Inhibit neuronal senescence.	P53↓, GSH↑, enhance glycolysis and inhibit mitochondrial metabolism to anti-cuproptosis.	[310-312]
Hypericum perforatum, Ginkgo biloba, and elderberry	Quercetin	Antioxidant; Activate autophagy.	Repaired the copper neurotoxicity.	[313, 314]
Camellia sinensis	Epigallocatechin gallate (EGCG)	Antioxidant; Anti-inflammatory; Improve mitochondrial function.	Binding to Cu (II) to reduce the Cu (II)-mediated toxicity.	[315, 316]
Carthamus tinctorius	Hydroxysafflor yellow A (HSYA)	Antioxidant.	Reducing copper-induced oxidative stress.	[317, 318]
Turmeric	Curcumin	Antioxidant; SIRT1↑; Modulating autophagy.	GSH↑, increased copper chelation and repaired the copper neurotoxicity.	[319-321]
Grape, blueberry, peanut	Resveratrol	Antioxidant; Anti-inflammatory; Improve mitochondrial function; Balance cellular proteostasis; Regulation of apoptosis.	GSH↑	[107, 322]
Chlorophytum borivilianum	C.borivilianum root extracts	Antioxidant; Suppressed the lipid peroxidation in mitochondrial fractions.	Copper chelation; Reducing copper-mediated oxidative damage.	[323]
Magnolia officinalis	Magnolol	Antioxidant.	Reducing copper-triggered LDL oxidation.	[324]
Coptis chinensis (Huanglian)	Berberine	Antioxidant; Anti-inflammatory; Upregulating longevity-related genes.	Reducing copper-mediated oxidative damage.	[325, 326]

Aβ, amyloid beta-peptide; GSH, glutathione; LDL, low density lipoprotein; P53, tumor Protein 53; ROS, reactive oxygen species.

7.4 Novel targeted strategies: nanomedicines and gene therapies

7.4.1 Nanomedicines

Nanomedicines have garnered considerable attention in recent years. Metallic nanoparticles, due to their unique electronic, optical, and physicochemical properties, are commonly used in applications such as photothermal therapy [301]. Copper nanoparticles (Cu NPs) possess natural availability and are used in the treatment of bacterial infections, delivery of drugs across the BBB [302], and as potential materials for tissue regeneration. Moreover, they are readily metabolized and cleared from the body. GCA-CD, a supramolecular inhibitor engineered from guanidinium-calixarene and cyclodextrin co-assembly, has been shown to disrupt α-synuclein fibrillation. It concurrently alleviates mitochondrial deficits, scavenges oxidative stress, and counteracts cuproptosis through the inhibition of FDX1/LIAS in PD rat models [303].

7.4.2 Gene therapies

In recent years, gene therapy has emerged as a treatment method that has attracted considerable attention [304]. It utilizes various vectors, including adenovirus vectors, lentiviral vectors, and adeno-associated virus (AAV) vectors, to insert normal functional genes into defective cells and treat genetic diseases caused by gene defects. Traditional gene therapy approaches carry the risk of disrupting valuable genes unrelated to the therapeutic

purpose. The gene editing tool CRISPR/Cas9 may offer a suitable opportunity to overcome this challenge, this gene editing technology modifies or inactivates specific genes to achieve the goal. CRISPR/Cas9 can perform genetic correction on ATP7B without affecting the expression of ATP7A, thereby improving copper efflux [305]. This represents a prospective treatment method for copper-related diseases. A single gene encoding the mitochondrial reductase FDX1, a key protein in cuproptosis, has been shown to rescue ES-induced cuproptosis [306]. Targeting the gene encoding ATP7B, which enables erroneous ATP7B to be as effective as normal ATP7B, holds the potential to correct copper efflux and mitigate cuproptosis [307, 308].

Table 3. Therapeutic strategies targeting cuproptosis: a comparative analysis of mechanisms, findings and prospects for clinical translation.

Therapeutic strategies	Representative drugs/ Methods	Mechanisms	Current primary research phase (model)	Findings	Potential advantages	Limitations
Copper chelators	D-penicillamine, trientine	Inhibition of detrimental cuproptosis through modulation of systemic or local copper levels.	Approved for WD; Preclinical studies (AD)	Well-established efficacy in WD; Demonstrated neuroprotective effects in AD.	Safety profile is relatively well-established; Suitable for the diagnosis of copper overload pathology.	Systemic copper depletion risk, potentially leading to anaemia and neurological symptoms [327].
Cuproptosis pathway inhibitors	ACT-132577, DEX	Direct inhibition of cuproptosis through an unknown mechanism.	Preclinical studies	Demonstrated neuroprotective effects in brain.	Directly acts on the pathological target, minimizing systemic disruption of copper homeostasis, and offers a novel avenue for drug repurposing.	The underlying mechanism remains unclear, and clinical evidence from human studies is currently lacking.
Natural medicines	Curcumin, quercetin	Multi-target activities: metal chelation, antioxidant and anti-inflammatory effects, and potential modulation of associated protein expression.	Preclinical studies	Demonstrated protective effects in multiple animal models of age-related diseases.	A favorable safety profile supporting long-term/preventive use; Pleiotropic efficacy aligned with complex pathophysiology; High tolerability and public acceptance [328, 329].	Unclear therapeutic utility and low bioavailability [330].
Nanomedicines	Functionalised nanoparticles loaded with chelators or natural products.	Facilitation of targeted drug delivery, including BBB penetration, for enhanced therapeutic efficacy.	Preclinical studies	Improved targeting biodistribution and therapeutic efficacy over the untargeted compound in animal studies.	Potential to overcome the dual challenges of low bioavailability in natural products and off-target toxicity in conventional pharmacotherapy [331].	Material complexity; absence of long-term toxicity data; A substantial translational gap to clinical application [332].
Gene therapies	Gene editing tool CRISPR/Cas9, AAV-ATP7B	Precision correction at the genetic origin or key nodal points of signaling pathways.	Preclinical studies	Long-term modulation of copper homeostasis.	Potential for curative intervention in monogenic disorders; the highest degree of precision [333].	Long-term safety and immunogenicity concerns, efficacy variability in aging and prohibitive costs [334, 335].

WD, Wilson's disease; AD, Alzheimer's disease; DEX, Dexamethasone; AAV, Adeno-associated virus; ATP7B, ATPase copper transporting beta gene.

While therapeutic strategies such as gene therapy, chelators and natural products hold strong conceptual promise, their translational potential differs markedly. To facilitate a systematic evaluation and comparison, Table 3 summarizes their mechanisms of action, current research stage, key findings, principal advantages, and limitations.

This comparison helps clarify the relative position of each strategy within the research landscape and can inform future research priorities.

8. Discussion

The precise relationship between cuproptosis and the aging process embodies a central and unresolved conceptual dichotomy: whether cuproptosis is a cause or consequence of cellular aging. As synthesized in this review, current evidence supports a dynamic, bidirectional model over a linear causal hierarchy, while also revealing critical knowledge gaps. Copper-related disorders can be viewed as both a consequence and an amplifier of the aging process. Aging is characterized by systemic and cellular decline, which includes impaired biliary and BBB copper excretion, reduced expression of copper-buffering proteins such as GSH and MT, and chronic inflammation. These changes promote pathological copper accumulation, and the resulting disruption of copper homeostasis renders mitochondrial metabolism vulnerable. In this context, cuproptosis may be regarded as a terminal clearance mechanism for cells already compromised by age-related metabolic stress, operating within a broader context of diminished biological resilience. Conversely, substantial evidence indicates that cuproptosis as a contributory cause that actively propels aging phenotypes. Activation of cuproptosis pathway not only leads to cellular loss, but copper overload and consequent cuproptosis trigger the release of mtDNA and HMGB1, thereby effectively activating cGAS-STING and other innate immune pathways. By generating a potent inflammatory response and SASP, cuproptosis creates a milieu that induces paracrine senescence, disrupts tissue microenvironments, and compromises stem cell function, thereby establishing a self-perpetuating cycle of cellular loss, inflammation, and accelerated tissue aging, accelerating the decline in biological resilience [336]. Critical knowledge gaps persist regarding the temporal dissociation between copper dysregulation and aging. It remains unclear whether copper overload and cuproptosis act as initiating drivers of cellular aging or as a consequence that clears damaged cells. To resolve this temporal ambiguity and distinguish initiating drivers from clearance consequences, future research must prioritize longitudinal analyses that correlate dynamic copper fluxes with the progression of aging biomarkers. A key priority will be to determine whether copper homeostasis imbalance consistently precedes other hallmarks of aging or emerges as a concurrent or secondary event. Additionally, what specific intracellular or mitochondrial copper concentration threshold is required to induce cuproptosis, and how does this threshold vary across tissues, cell types, and during the aging process? Emerging experimental evidence underscores the striking tissue-specific variability of such thresholds, yet consistent standards are lacking. In *Tsvetkov*'s foundational work, the threshold for triggering cuproptosis has been clearly demonstrated in human cell lines dependent on mitochondrial respiration, such as the melanoma cell line ABC1. A 2-hour pulse treatment with the copper ionophore ES at concentrations as low as 40 nM leads to a 15-60-fold increase in intracellular copper levels and subsequent cell death after 24 hours. This process depends on high levels of lipoylated proteins and is particularly sensitive in cells that rely metabolically on the TCA cycle, whereas cells primarily dependent on glycolysis exhibit resistance [8]. In a rat model of cholestasis, a hepatic copper concentration ≥0.68 μmol/g tissue (approximately 1.4-fold above baseline) was associated with downregulation of key cuproptosis markers (FDX1, DLAT, DLST, LIAS) and liver injury. Chronic copper accumulation reaching 1.07 ± 0.2 μmol/g correlated with progressive fibrosis and sustained cuproptotic features. In pediatric patients with cholestasis, serum copper levels >16.3 μmol/L were linked to decreased FDX1 expression in hepatocytes. *In vitro*, treatment of rodent hepatocyte lines (BRL 3A and AML12) with $CuCl_2$ (150–300 μM) combined with the copper ionophore ES (40 nM) for 24 hours consistently recapitulated the characteristic molecular signature of cuproptosis, confirming the defined concentration thresholds [337]. Cardiac cells exhibit yet another sensitivity profile. In the diabetic cardiomyopathy model, in *vitro* studies utilizing human cardiomyocyte lines (AC16, H9c2, HL-1) demonstrated that cuproptosis could be triggered at a threshold of 10 μM $CuCl_2$. Significant cardiomyocyte death was observed when this was combined with copper ionophores such as 40 nM ES or with AGEs at 100 μg/mL [241]. In addition, the selectivity of cell types is observed in neural tissue. In primary mixed glial cultures treated with the copper ionophore ES, astrocytes exhibited marked susceptibility to ES induced toxicity, whereas neurons remained strikingly resistant. Specifically, astrocyte-only cultures showed significant cell death at concentrations as low as 2 μM Cu^{2+} and 50 nM ES. In contrast, cocultures containing both neurons and astrocytes required higher thresholds, namely 3 μM Cu^{2+} and 200 nM ES, to elicit comparable toxicity, suggesting a potential neuroprotective role of neurons toward adjacent astrocytes [338]. In human neurons cultured *in vitro*, 100 μM $CuSO_4$ for 48 hours disrupted both the lysosomal and mitochondrial networks, representing the lowest effective concentration for mitochondrial-targeted toxicity. At 420 μM, lysosomal integrity was completely lost, oxidative stress emerged as a subsequent event at higher concentrations (>400-500 μM) [339]. Although the precise copper toxicity

thresholds for each cell type remain undetermined, variations exist between different cell types: in acute 24-hour exposure experiments using human hepatoma (Bel-7402) and renal tubular epithelial (HK-2) cells, human hepatoma cells displayed significantly greater sensitivity to copper ions than renal epithelial cells [340]. While cuproptosis requires a 15–60-fold intracellular copper surge, a mild 1.5–2-fold mitochondrial copper increase in fibrotic mouse kidneys and TGF-β1-stimulated rat tubular cells NRK-52E is sufficient to induce lipoylated DLAT dimerization, inhibit PDH activity, and drive cellular senescence and fibrosis without triggering acute cell death [108]. These findings illustrate that cuproptosis thresholds are not uniform but are shaped by cellular metabolism, baseline copper-handling capacity, tissue microenvironment, and the presence of interacting cell types. While acute cuproptosis in cancer cells occurs with nanomolar ES and dramatic copper amplification, parenchymal cells in the liver, heart, and brain exhibit higher or more variable thresholds, and subtler copper rises may suffice to drive non-lethal lipoylated protein remodeling and chronic disease progression. However, the field still lacks systematic comparative studies that directly measure cuproptosis thresholds across different cell types or tissues under identical experimental conditions. This gap is not simply due to insufficient research but rather highlights the intrinsic complexity of cuproptosis regulation, which is deeply intertwined with cell-type-specific copper metabolism machinery and age-related physiological remodeling. Most studies fail to distinguish between total copper and unstable copper pools, it is specifically the unbound, labile copper that initiate cuproptosis via targeting FDX1-mediated protein lipoylation. Secondly, Aging remodels threshold patterns through cellular altered copper buffering capacity and impaired mitochondrial resilience: altered copper buffering capacity and impaired mitochondrial resilience. In aged cardiomyocytes, the downregulation and mislocalization of ATP7A impair cellular copper export. This may not alter the intrinsic threshold for cuproptosis. Nevertheless, the concomitant decline in copper-buffering proteins means that even moderate external copper exposure can now drive intracellular concentrations to toxic levels. In a parallel manner, aging neurons and osteoblasts exhibit a reduced mitochondrial membrane potential, altered permeability and impaired mitochondrial resilience, these deficits weaken mitochondrial adaptive capacity and enhanced cuproptosis [52, 341, 342]. Moreover, *in vivo*, the systemic buffering capacity of copper-homeostatic networks, including CP-mediated transport, mitigates local copper variations, meaning thresholds measured in isolated cells may overestimate *in vivo* sensitivity. Thus, copper concentration and cellular responsiveness become increasingly decoupled with advancing age highlights the imperative to advance beyond static threshold models and adopt dynamic, real-time measurement paradigms. Defining the cuproptosis threshold requires consideration of not only the free copper concentration but also tissue metabolic profiles, age-related changes, and the broader physiological context of whole-body copper homeostasis. The interpretation of the mechanistic links discussed in this review is inherently restricted by the experimental models on which current evidence relies. Current understanding relies heavily on rodent studies, although valuable for elucidating systemic copper metabolism and aging phenotypes, these models have limited translational relevance to human pathophysiology. Interspecies variations in copper homeostasis, lifespan, and disease manifestation limit the applicability of rodent data to human aging and related disorders [343-345]. At the cellular level, the foundational mechanisms of cuproptosis have been elucidated primarily *in vitro*, utilizing cancer cell lines or short-term cultures. While these studies established the core FDX1-lipoylation axis, they may not fully recapitulate the slow, cumulative copper dyshomeostasis and the integrated stress responses within post-mitotic, aged cells *in vivo*, such as neurons or cardiomyocytes. Moreover, the susceptibility to cuproptosis varies markedly across cell types, as evidenced by differing toxicity thresholds between hepatic and renal epithelial cells *in vitro*, highlighting the context-dependency that simplified models cannot entirely capture. Therefore, while existing models have been instrumental in discovering and dissecting cuproptosis, bridging the gap between these mechanistic insights and human aging will require the development of more physiologically relevant models, such as human organoids, aged animal models with targeted genetic manipulations, and longitudinal studies integrating biomarkers of copper flux with aging hallmarks in humans.

9. Conclusion and future perspective

Copper, an essential trace element and well-established enzymatic cofactor, can accumulate under various pathophysiological conditions. Beyond its conventional role in mitochondrial respiration and antioxidant defense, Copper accumulation induces a novel RCD, termed cuproptosis. Cuproptosis is mediated by mitochondrial ferredoxin 1-mediated protein lipoylation, excess copper ions interact with lipoylated proteins, resulting in protein oligomerization, and obstruct the FDX1-mediated synthesis of Fe-S cluster proteins, resulting in Fe-S cluster proteins destabilization, GSH protein depletion, and the induction of proteotoxic stress, ultimately leading to cell

death. This review synthesizes compelling evidence that copper homeostasis imbalance and cuproptosis are integral to the biology of aging and the pathogenesis of age-related diseases. Aging perturbs systemic and cellular copper regulation through mechanisms involving impaired excretion, barrier dysfunction, reduced buffering capacity, and chronic low-grade inflammation, leading to a progressive accumulation of redox-active copper. This copper overload, in turn, drives cellular damage through both direct toxic effects and cuproptosis. We have delineated how this pathway interfaces with hallmarks of aging, including mitochondrial dysfunction, proteostasis imbalance, epigenetic alterations, and chronic inflammation, thereby creating a pathogenic feedback loop that accelerates functional decline. The relationship between cuproptosis and aging is complex and bidirectional. While an aging milieu promotes copper dyshomeostasis and susceptibility to cuproptosis, the execution of cuproptosis itself can amplify aging phenotypes through cell loss and the potentiation of sterile inflammation via DAMPs and the cGAS-STING pathway. This interplay is also observed in specific age-related pathologies, including neurodegenerative diseases, cardiovascular disorders, T2DM and OP, where modulation of copper levels or cuproptosis components has shown therapeutic potential. Significant knowledge gaps remain, particularly concerning the spatiotemporal dynamics of cuproptosis *in vivo*, its cell-type specificity, and its precise causal role within the sequence of aging events. The elucidation of cuproptosis as a component of the aging process opens several critical research trajectories that extend from fundamental mechanisms to translational applications. A primary focus should be the spatiotemporal mapping of cuproptosis activation within aging organisms, utilizing advanced *in vivo* imaging and sensors to delineate its timeline and tissue specificity relative to other hallmarks of aging. Concurrently, expanding the molecular network beyond the core FDX1-lipoylation axis is essential, we should search for novel regulators and modulators, particularly those influenced by age-related signals, will reveal how broader metabolic changes integrate with this cell death pathway. In translational research, developing specific biomarkers of cuproptosis activity for patient stratification and designing precision therapeutic interventions such as targeted nanomedicine or gene therapy that can modulate this pathway without disrupting essential copper-dependent physiology are critical priorities. Ultimately, future research can transform cuproptosis from a novel cell death pathway into a leverage point for diagnosing and intervening in the aging process itself.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Jingyan Hong: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. Zhigang Mei: Writing – review & editing, Supervision, Conceptualization. Guozuo Wang: Writing – review & editing, Supervision, Funding acquisition. Yaxin Liu and Quan Peng: Reference collection and analysis.

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