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REVIEW

Copper in cancer: friend or foe? Metabolism, dysregulation, and therapeutic opportunities

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List of abbreviations: AD, Alzheimer's disease; AIFM1/AIF, apoptosis inducing factor mitochondria associated 1/apoptosis inducing factor; ALDH, aldehyde dehydrogenase; AMPK, AMP-activated protein kinase; ATK, apoptosis tyrosine kinase; Atox1, antioxidant 1 copper chaperone; BCL, B-cell lymphoma; BRCA, breast cancer; Cco, cytochrome C oxidase; CCS, cytochrome c oxidase copper chaperone; CDKN2A, cyclin-dependent kinase inhibitor 2A; CDT, chemodynamic therapy; CNV, copy number variation; COMMD10, copper metabolism MURR1 domain 10; COX, cytochrome C oxidase; COX17, cytochrome C oxidase17; CP, ceruloplasmin; CRC, colorectal cancer; CRIP2, copper response element-binding protein 2; CSCs, cancer stem cells; CTR1, copper transporter 1; Cu3BiS3, Cu- and Bi- based bimetal chalcogenide; Cu-Cy, cupro-cysteamine; CuET, copper-containing metabolites; DBH, dopamine beta-hydroxylase; DBT, dihydrolipoamide branched chain transacylase; DDR, DNA damage response; DDTC, diethyl dithiocarbamate; DLAT, dihydrolipoamide acetyltransferase; DLST, dihydrolipoamide S-acetyltransferase; DMT1, divalent metal transporter 1; DSF, disulfiram; ERK, extracellular signal-regulated kinase; ERK, extracellular signal-regulated protein kinase; ES, elesclomol; FABP3, fatty acid binding protein 3; FABP7, fatty acid binding protein 7; FDX1, ferredoxin 1; FGF, fibroblast growth factor; GCSH, glycine cleavage system H protein; GPX, cystathione peroxidase; GPX4, glutathione peroxidase 4; GSH, glutathione; GST, glutathione-S-transferase; HCC, hepatocellular carcinoma; HD, Huntington's disease; HIF1, hypoxia-inducible factor 1; HIF1α, hypoxia-inducible factor 1 subunit α; HMGB1, high-mobility group box 1; HNSCC, head and neck squamous cell carcinoma; HTT, huntingtin; HZIFCu, Cu^{2+}-doped hollow zeolite imidazoline framework nanoparticles; IGF, insulin-like growth factor; IL-1α, inflammatory cytokine; IL-1β, interleukin-1 beta; IMS, mitochondrial membrane space; KIRC, kidney renal clear cell carcinoma; LA, lipoic acid; LDH, lactate dehydrogenase; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; MAP2K1, mitogen-activated proteinkinase kinase 1; MAPK, mitogen-activated protein kinase; MBDs, metal-binding domains; MEK, mitogen-activated proteinkinase kinase; MEK1, mitogen-activated protein kinase 1; MICOS, mitochondrial contact site and cristae organizing system; MLKL, mixed lineage kinase domain-like; MOF, metal-organic framework; MT, metallothionein; NRCP, lncRNA cuprocyanin; NSAID, nonsteroidal anti-inflammatory drug; P38MAPK, p38 mitogen-activated protein kinase; PANoptosis, pan-apoptosis; PCD, program cell death; PDE3B, phosphodiesterase 3B; PD-L1, programmed death-ligand 1; PET, positron emission tomography; RIPK1, receptor-interacting serine/threonine-protein kinase 1; RIPK3, receptor-interacting serine/threonine-protein kinase 3; ROS, reactive nitrogen species; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; sCP, serum CP; SKCM, skin cutaneous melanoma; SOD, superoxide dismutase; STEAP, six-transmembrane epithelial antigen of prostate; TCA, tricarboxylic acid; TGN, trans-Golgi network; TM, tetrathiomolybdate; TMZ, temozolomide; TNBC, triple-negative breast cancer; UPS, ubiquitin-proteasome system; VEGF, vascular endothelial growth factor; ZnT1, zinc transporter 1.

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Abstract

Copper, one of the essential nutrients for the human body, acts as an electron relay in multiple pathways due to its redox properties. Both deficiencies and excesses of copper lead to cellular fragility. Therefore, it can manifest pro- and anti-cancer properties in tumors. Therefore, it is crucial to clarify the copper activity within the cell. We have thoughtfully summarized the metabolic activities of copper from a macro and micro perspective. Cuproptosis, as well as other forms of cell death, is directly or indirectly interfered with by Cu^{2+}, causing cancer cell death. Meanwhile, we did pan-cancer analysis of cuproptosis-related genes to further clarify the roles of these genes. In addition, copper has been found to be involved in multiple pathways within the metastasis of cancer cells. Given the complexity of copper's role, we are compelled to ask: is copper a friend or a foe? Up to now, copper has been used in various clinical applications, including protocols for measurement of copper concentration and bioimaging of radioactive ^{64}Cu. But therapeutically it is still a continuation of the old medicine, and new possibilities need to be explored, such as the use of nanomaterials. Some studies have also shown that copper has considerable interventional power in metabolic cancers, which provides the great applications potential of copper therapy in specific cancer types. This paper reviews the dual roles played by cuproptosis in cancer from the new perspectives of oxidative stress, cell death, and tumor metastasis, and points out the value of its application in specific cancer types, summarizes the value of its testing and imaging from the perspective of clinical application as well as the current feasible options for the new use of the old drugs, and emphasizes the prospects for the application of nano-copper.

KEYWORDS
cancer therapy, copper metabolism, cuproplasia, cuproptosis, intracellular copper-associated pathway

1 | BACKGROUND

As a common nutrient metal, copper is abundant in vegetables, mushrooms, legumes, and whole grains [1]. There is a cycle of the element copper in nature, and similarly, there is a flow of copper in the human body, which we call copper metabolism, which differs in that the form of existence is ionic. The activities of copper in the human body encompass several processes, including uptake, transportation, distribution, utilization, storage, and excretion. Macroscopically, copper is absorbed in the small intestine, transported by blood, stored in tissue cells, and excreted primarily through bile, with less than 5% being excreted through urine. In the blood, copper is bonded to ceruloplasmin (CP) and then distributed to various tissues throughout the entire body [2]. In the histiocytes, copper participates in different pathways to achieve physiological functions through various copper chaperone proteins. Microscopically, copper enters the interior of the cell through passive transport, aided by the copper transporter protein copper transporter 1 (CTR1) [3], where it undergoes an electrochemical process [4, 5]. Intracellularly, copper benefits from its redox properties to act as a mediator of electron transfer in cellular pathways. For instance, copper binds to superoxide dismutase (SOD) to regulate enzyme activity, which is the key to the enzyme's antioxidant function [6]. In addition to this, there are cytochrome C oxidase (COX), dopamine beta-hydroxylase (DBH), phosphodiesterase 3B (PDE3B), antioxidant 1 copper chaperone (Atox1) among others. Ultimately, concentration-driven ATPase copper transporting alpha/beta (ATP7A/B) mediates the release of copper ions from copper-containing vesicles to accomplish copper excretion [7, 8]. The human body regulates copper concentrations inside and outside of the cell membrane via copper metabolism-related protein, achieving

copper homeostasis. Copper homeostasis is defined as the state in which a cell remains relatively stable in its intracellular environment through activities such as ingestion and excretion. These copper metabolism-associated proteins constitute a complex network of protein interactions, and when the positions of these anchors are misplaced, they can cause disturbances in physiological functions such as energy metabolism, cell growth, proliferation and migration, angiogenesis, and neurodevelopment [9]. In fact, cancers such as prostate, breast, thyroid, stomach, and lung cancers, which are concentrated in metabolic cancers, also show a strong copper correlation [10]. Copper is a trophic factor as well as one of the rate-limiting factors for the growth and proliferation of cancer cells. Conversely, copper can act as an inducer of oxidative stress and cell death [11]. Two diametrically opposed concepts, cuproplasia and cuproptosis, reveal the bidirectional role of copper in cancer. Drugs based on copper metabolism have been found to be promising in recent years for breaking through conventional chemotherapy-resistant cancers and have shown promising cancer inhibition effects in neoadjuvant radiotherapy and chemotherapy [12]. Is copper our friend or foe? This review will identify specific mechanisms of copper metabolism and elaborate on those with cancer therapeutic potential, thus opening clinical perspectives on copper.

2 | COPPER METABOLISM AND HOMEOSTASIS

Copper homeostasis is defined as a relatively stable state maintained through the regulation of fixed and free copper levels in the cytoplasm, achieved via processes of uptake and excretion. In contrast, copper imbalance refers to a state characterized by abnormally high, low, or unevenly distributed copper levels throughout the body. Copper homeostasis is maintained by the copper-metabolism-related proteins, including cuproenzymes, copper chaperones, and membrane transporters. Under the protein network composed of copper metabolism-related proteins, copper undergoes transfer between the fixed and dissociative pools, achieving a dynamic equilibrium in response to various diseases resulting from copper overload or deficiency [13]. Summarizing the specific mechanisms of copper metabolism can help to construct a comprehensive landscape of copper, thereby providing a theoretical basis for copper-based therapies in cancer. This part makes a summary of the processes related to copper metabolism, containing copper uptake, and copper chaperone-dependent intracellular copper-associated pathways of copper and copper excretion (Figure 1).

2.1 | Copper uptake

In the digestive system, Cu(II) cannot be directly absorbed by the small intestine, oppositely reduced to Cu(I) via the six-transmembrane epithelial antigen of prostate (STEAP) family of metal reductases before entering into the cell [4, 14]. CTR1 is the main transporter of copper ions, and more than eighty percent of copper transport relying on this pathway [15]. In addition to this, Divalent metal transporter 1 (DMT1) and anion transporters also facilitate copper transport [16]. CTR1 is composed of 190 amino acids with several metal binding sequences [17]. According to experiments by Eisses et al. [18] have shown that binding copper ions to proteins during transport induces conformational changes. The binding of copper ions to the His-Cys-His sequence allows the entry of copper ions into the protein's pore, guided by the sequence of Met residues closest to the membrane in the amino terminus of CTR1, forming a stacked ring [17, 19]. Notably, the presence of the His-Cys-His sequence itself retards the transport of copper ions [20], which may offer a potential target for research aimed at regulating the rate of copper ion transport. Well known as a transporter protein for iron ions, DMT1 also transports copper ions. Previous studies have demonstrated experimentally that copper and iron transport possibly via DMT1 are correlated [16, 21]. Apart from the above, copper can also be transported as metal-ion complexes of the $[MnCl]_n^{2-}$ or $[MClOH]_n^{1-}$ types [22]. Recent studies have shown that zinc transporter 1 (ZnT1) pumps copper into cells while excreting zinc ions and has an essential role in cuproptosis [23–25]. It is suggested that our in-depth analogy to the metabolic activities of divalent metals may lead to further clarity on the intracellular activities of copper [23].

2.2 | Copper chaperone-dependent intracellular copper-associated pathways

Once entering the cell, copper plays an active role in various intracellular pathways related to copper, leveraging its redox properties. This process is facilitated by a group of copper chaperone proteins, such as cytochrome C oxidase17 (COX17) [26, 27], cytochrome c oxidase copper chaperone (CCS) [28], and Atox1 [14, 28]. Additionally, due to the toxicity of free copper ions, metallothionein (MT) [29] and glutathione (GSH) [30] constitute an endogenous intracellular barrier to defend it.

COX17, a 69-residue protein, functions as a cytochrome C oxidase (Cco) copper chaperone primarily localized in the cytoplasm and mitochondria [31]. Its role involves facilitating the transport of copper ions from the cytoplasm to

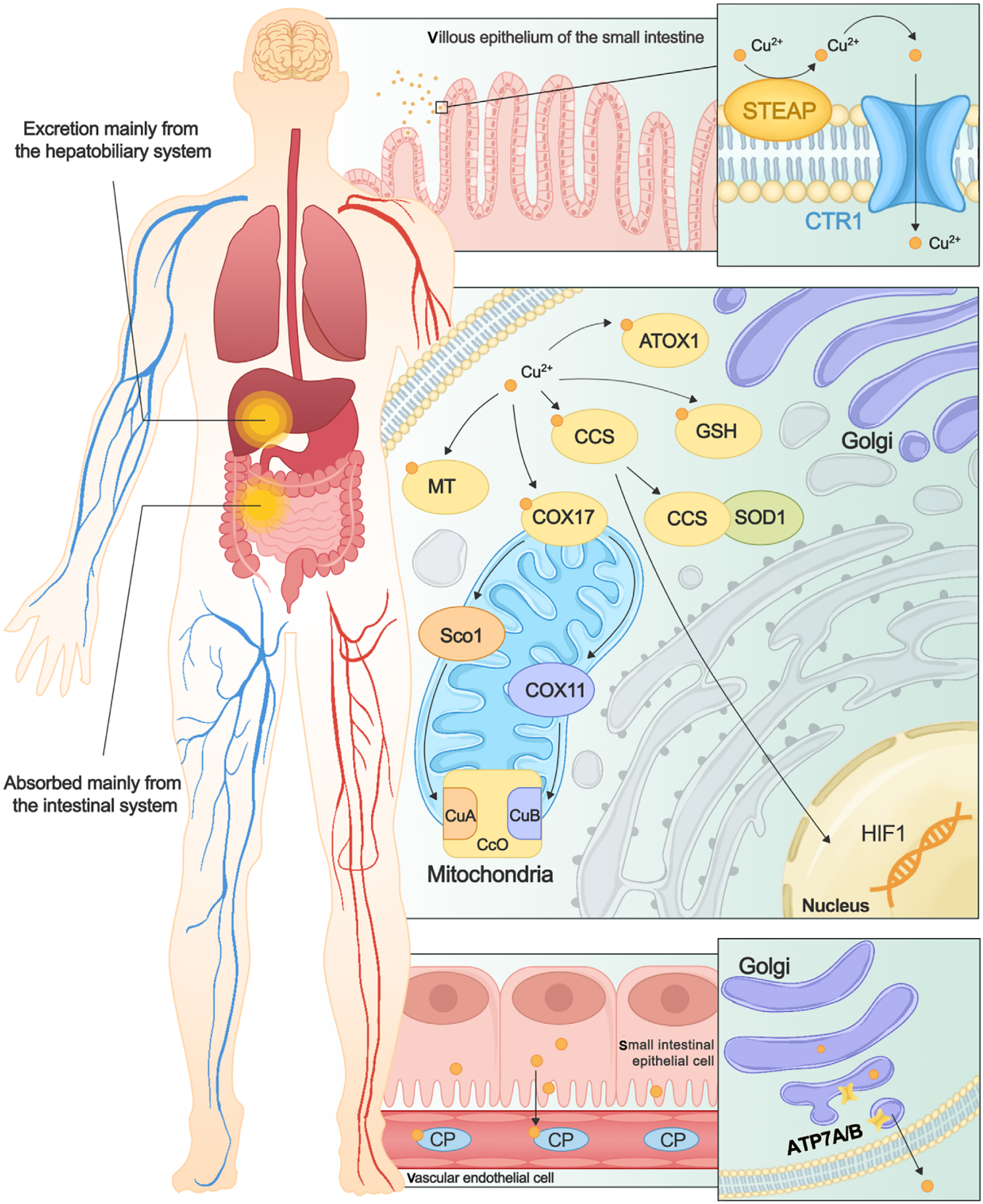

FIGURE 1 Copper metabolism in macro and micro perspective. Copper metabolism has been summarized into three components, uptake, excretion and intracellular copper metabolism. In the digestive tract, copper exists as a divalent ion, which is reduced by STEAP proteins outside the cell membrane and enters the cytosol via transmembrane proteins, such as CTR, and is then taken up by small intestinal epithelial cells. Intracellularly, CCS is manipulated by copper to participate in the activation of SOD1 and the gene HIF1. Copper transported into the mitochondria by COX17 is involved in the respiratory chain by activating the CuA and CuB sites of Cco via the Sco1 or COX11

the mitochondria, where they are utilized in redox processes as components of Cco [14, 32, 33]. Cco is the main site of ATP production by aerobic respiration and is mainly composed of two subunits, Cox1 and Cox2 [34]. Cobine et al. [32] using yeast, demonstrated that the activity of Cco is dependent on COX17. Recent experiments have further demonstrated that the manipulation of Cco's activity can be achieved through copper ions [35]. In mitochondria, Cco obtains copper through two pathways. On one hand, COX17 delivers copper ions to Sco1 [36] and Sco2 [37], proteins in the inner mitochondrial membrane, which deliver copper to the CuA site of subunit COX2. On the other hand, COX17 delivers copper to COX11, which in turn delivers copper to the CuB site of the subunit COX1 [32, 33, 38]. Besides, the mitochondrial contact site and cristae organizing system (MICOS) has recently been found to be essential for the maintenance of mitochondrial membrane structure, while copper plays a role in facilitating the regulation of the MICOS complex by Cox17 [38].

As copper chaperones, CCS sends copper to the specific protein SOD1 [39] in the cytoplasm as well as it sends copper to the nucleus to regulate the transcription factor *HIF1* [40]. SOD1(Cu/Zn-SOD) is a homodimeric enzyme, abundant in the cytoplasm, CCS captures Cu from CTR1 and forms the "CTR1-CCS-SOD1 complex", which spontaneously dissolves once Cu activates SOD1 [41]. With the help of the metal cofactor copper, SOD1 exploits its redox properties to neutralize superoxide compounds and protect cells from oxidative damage [42]. Numerous experiments have shown that the deletion or mutation of SOD1 increases oxidative stress in vivo [39, 43–45]. Furthermore, a small fraction of SOD1 localizes to the mitochondrial membrane space (IMS), in which CCS participates [46, 47]. CCS can also transport copper into the nucleus to activate and mediate the up-regulation of the *HIF1* gene, thereby inhibiting lipid peroxidation and ferroptosis [14].

The copper chaperone Atox1 is an intracellular soluble protein that acts as a copper transporter between the cytoplasm and the Golgi apparatus, assisting in the efflux and distribution of copper [33], holding antioxidant [48, 49] and pro-proliferative effects [50]. The carrier function of Atox1 is dependent on the metal-binding region residues in its crystal structure including Lys65 [48]. After successful docking of copper ions, Atox1 delivers them to the Golgi copper-associated transporter proteins Ccc2/ATP7A/B, and these grab copper ions into the Golgi with the help of the amino-terminal 2-6 MxCxxC metal-binding domains (MBDs) [51–54]. Arnesano et al. [55] utilized HADDOCK to establish a structural model of the transient complex between the copper chaperone, Atox1, and the first soluble structural domain of the ATPase Ccc2 demonstrating the copper transport process. Therefore, we hypothesized that targeting Atox1 could inhibit copper excretion via an indirect pathway.

Free copper ions are toxic within the cell [14], and the cell itself has a counterpart remedy in the form of sending MT and GSH to bind to copper ions. MT is a protein family capable of intracellular metal binding, with four major isoforms (I-IV). MT-I and MT-II, multivalent proteins comprising 61-68 residues, including cysteine residues, are primarily located in astrocytes of the spinal cord and brain. The two isoforms play a crucial role in maintaining homeostasis of copper, zinc, and other metals. The thiol group in cysteine is integral to MT's function, chelating copper ions released from Ctr1 [56]. GSH is a tripeptide consisting of glycosidic acid, cysteine, and glycine residues [57], which is localized in all mammalian tissues and highly concentrated in liver tissue [58]. At the cellular level, 90% of GSH is in the cytoplasm, with the rest distributed in the endoplasmic reticulum and mitochondria [59, 60]. Intracellular GSH can bind electrophilic substances such as copper ions [58] prior to MT, and form affixes catalyzed by GSH-S-transferase to achieve detoxification. Regulating the concentration of MT and GSH in specific cells might induce cell death by increasing the toxicity of free copper.

2.3 | Copper excretion

Menkes' disease, which is also called copper deficiency disease, and Wilson's disease, which is characterized by intrahepatic copper accumulation, are both genetically defective diseases. Studies of the genes in them have revealed important players in copper efflux: ATP7A [61] and ATP7B [62, 63].

The distribution of Cu-ATPase is physiologically crucial for copper excretion and coordination. At the organ level, ATP7A is found in most organs and tissues with the exception of the liver, where it facilitates the import of

pathways, respectively. Excess free copper is neutralized by MT with GSH. Copper transport is realized by ATOX1 trapping it to the Golgi via ATP7A/B-driven cytosolization of the Golgi in association with the cell membrane. It is transported in the bloodstream bound to copper cyanoproteins and eventually excreted in bile in conjunction with urine. Abbreviations: ATOX1, human antioxidant protein 1; CcO, cytochrome c oxidase; CCS, cytochrome c oxidase copper chaperone; COX11, cytochrome c oxidase11; COX17, cytochrome c oxidase17; CP, ceruloplasmin; CTR1, copper transporter 1; GSH, glutathione; HIF1, hypoxia-inducible factor 1; MT, metallothionein; Sco1, small mitochondrial copper binding protein 1; SOD1, superoxide dismutase 1; STEAP, six-transmembrane epithelial antigen of prostate.

copper ions into the circulation system from the intestinal epithelium, and these copper ions can be subsequently reabsorbed by hepatocytes [64]. ATP7B is expressed predominantly in hepatic and neuronal tissues, and intrahepatic ATP7B allows the excretion of copper ions via the bile [7, 65]. At the organelle level, copper excretion involves the coordinated action of ATP7A or ATP7B proteins with the Golgi apparatus and cell membrane. The signal that triggers the copper efflux behavior of both is the copper concentration. When the intracellular copper concentration is at a low basal level, both are localized in the trans-Golgi network (TGN). In response to high intracellular copper concentrations, ATP7A and B are translocated to intracellular vesicles, where they act as transport vehicles to sequester copper and deliver it to post-Golgi vesicles, dependent on the characteristics of plasma membrane fluidity [8, 66–68]. At the molecular level, the molecular mechanism of copper transport by ATP7A and ATP7B may depend on the proximity of amino acid regions to the MBDs of the channel [42]. Studies of copper utilisation may reveal additional therapeutic perspectives for refractory cancers. Recent studies have shown that in colorectal cancer (CRC), ATP7A can effectively target oncogenic KRAS and exert a protective effect on cells, bringing new hope for the treatment of cancers with KRAS vulnerability [69].

3 | OXIDATIVE DAMAGE CAUSED BY COPPER IMBALANCE INCREASES THE POSSIBILITY OF CELLULAR CARCINOGENESIS

Reactive oxygen species (ROS) are a class of oxygen-containing derivatives, mainly including superoxide (O_2^-) and hydrogen peroxide (H_2O_2) [70]. Once viewed as merely toxic by-products, they are typically found in subcellular locations such as mitochondria, the endoplasmic reticulum, and cell membranes, where there is high electron transport activity [71].The overflow of ROS induces oxidative stress, which in turn induces oxidative damage including DNA damage [70], stimulates cellular activities such as autophagy, and has macroscopic physiological effects on the human body, such as aging and cancerous transformation [72]. However, recent studies have found that the physiological effects of ROS on cells are not only negative. Dickinson et al. [71] summarized in his article that in addition to affecting cell proliferation and migration, the physiological effects of ROS may also have a regulatory effect on circadian rhythms and neurodevelopment, and the free radical theory of aging has been challenged [72], breaking the image of ROS as a consistent toxic factor in the human body.

In studies of cancer cell metabolism, it has been recognized that overproduction of ROS always accompanies cancer [73–75]. Downstream responses to oxidative stress, such as cell proliferation and survival, DNA damage and genetic instability, autophagy and cell death, and adaptation and drug resistance, are thought to be key factors leading to cellular carcinogenesis [76]. In contrast, metallic copper is involved in the elimination of ROS mediated by copper chaperones and occupies an important position in the complex regulatory network of ROS [77]. Copper chaperones like COX17, CCS, Atox1, GSH, etc. directly or indirectly realize the regulation of ROS by copper and present different subcellular localizations according to where ROS is produced [14, 78, 79].

Corresponding to the physiological functions of ROS, it's effect on tumors is also bidirectional. Elevated ROS cause carcinogenesis, while excessive ROS concentrations induce tumor cell death [80]. But in most cases, the mechanism of association between ROS and copper is where the underlying logic of copper-induced cell death lies. In-depth analysis of the specific mechanisms of copper in oxidative stress can help optimize the bidirectional role of ROS in cancer therapy, and copper is expected to be an effective regulatory target for ROS.

3.1 | Copper imbalance causes oxidative damage

The vast majority of ROS are produced in the heart of aerobic respiration, the mitochondria, in which ROS neutralized mainly by SOD and COX. There are three family members of SOD, SOD1 (CuZnSOD), SOD2 (MnSOD) and SOD3 (EcSOD), which are mainly located in the cytoplasm or mitochondrial membrane space, in the mitochondrial matrix and extracellularly, respectively [81–83]. A gradient copper concentration was set in chicken liver cells, and it was found that excess copper treatment was accompanied by an increase in ROS and SOD. It was speculated that copper could induce oxidative stress caused by an increase in ROS to some extent [84].While SOD2 (MnSOD) exerts a major antioxidant role [85], less attention has been given to the fact that SOD1 is activated by copper to convert superoxide to H_2O_2, also exerts an anti-oxidative stress role [86, 87]. Another SOD member with copper engaged, SOD3, scavenges reactive nitrogen species (RNS) in addition to ROS outside the cell, and is a class of superoxide that has been less discussed in the relevant literature. COX is involved in constituting the last link of the respiratory chain within mitochondria [88]. A particular strain, Sclerotinia sclerotiorum, was found to be entirely dependent on ROS regulation for its development on regulating ROS for development. Overexpression of the SsCox17 gene by

the designer strain revealed an increase in copper content and enhanced tolerance to oxidative stress, suggesting that the presence of COX17 positively correlates with the neutralization of ROS toxicity [89].

A variety of copper chaperones are also present in the cytoplasm, regulating intracellular oxidative stress processes while participating in copper metabolism. Atox1, which plays a role in copper transport within the copper metabolic system, was originally named for its antioxidant effects [90–92]. The Cys thiol residue in copper chaperones such as Atox1 is the main site for liganded Cu(I), which was found to be reversibly oxidized, making Atox1 key to inhibiting intracellular oxidative stress [93, 94]. The glutathione family contains a series of enzyme systems, including GSH, cystathione peroxidase (GPX), and glutathione-S-transferase (GST), which play important roles in redox systems. Among them, GSH can reduce copper-derived ROS production by chelating free copper ions [95], in addition to reducing disulfide bonds in the cytoplasm [96]. Under hypoxic conditions, *HIF1* acts as an upstream-regulated transcription factor [73], inducing GSH transcription and inhibiting copper-dependent mitogen-activated proteinkinase kinase 1 (MAP2K1) activity.

3.2 | Oxidative stress and cancer

ROS homeostasis is an essential for cells to carry out normal physiological functions and signaling. Disruption of this balance can trigger inflammatory responses and potentially lead to cancer. The toxicity of ROS is mainly reflected in oxidative stress including DNA damage as well as DNA damage response (DDR) [70]. Oxidative stress contributes to cancer development mainly by altering the expression of oncogenes and tumor suppressor genes with the help of epigenetic modifications, non-coding RNAs, and transcription factors [97].

Although previous studies of Cu/ZnSOD have concentrated on neurodegenerative diseases such as ALS, it has been shown that SOD1 knockout mice can also die from liver tumors [87]. In addition, SOD1 overexpression has been detected in various cancers [86]. The study by Somwar et al. [98] identifies SOD 1 as a potential target for small molecule inhibitor screening in lung cancer. Paradoxically, SOD3 expression is prevalent in most types of cancer (including breast, head and neck, lung cancers, etc.) [81]. The different SOD targets suggest that copper may perhaps exhibit a bidirectional regulatory role in cancer therapy. The elevated ROS produced by COX are also implicated in cancer development. Back in the last century, the use of the nonsteroidal anti-inflammatory drug (NSAID), a COX inhibitor, was found to be accompanied by a reduced risk of several cancers, including breast, bladder, and gastric cancers [99]. Recently, a research group discovered that the copper metabolism-associated protein COX17 is involved in the curative effects of the copper chelator tetrathiomolybdate (TM) on high-risk triple-negative breast cancer (TNBC) [27]. Whole blood transcriptional profiling indicates that Atox1 levels are altered in myelofibrosis and related tumors to increase antioxidant effects against tumors [100]. Furthermore, the team's study of the autophagy inhibitory protein copper response element-binding protein 2(CRIP2) revealed that it acts as a link in copper metabolism and regulates autophagy activation in the nucleus of cancer cells [101]. Chemotherapy in TNBC has been found to increase intracellular GSH through HIF-1, and the binding of GSH and copper initiates the inhibition of mitogen-activated proteinkinase kinase (MEK)-extracellular signal-regulated kinase (ERK) signaling pathway to achieve chemotherapy efficacy [102].

As an important metal cofactor that initiates the "copper-reactive oxygen species-oxidative stress-cancer" cascade, copper itself and its associated copper chaperones may serve as important targets for cancer regulation.

4 | CROSSTALK IS PRESENT AMONG COPPER-INVOLVED CELL DEATH PATHWAYS AND WIDELY ASSOCIATED WITH TUMORS

As a transition metal, the electronic structure of copper allows it to participate in intracellular pathways as a mediator of electron transfer, and similarly, iron possesses a similar function. In the 1980s, Halliwell et al. [103] in their study of cellular oxidative toxicity showed that copper and ferroptosis can produce OH- via the Fenton reaction and cause cell death via oxidative stress. It was surprising to find not only functional similarities between copper and iron, but also a considerable intersection of metabolic pathways in vivo [14]. Cytotoxicity caused by increased copper and iron is often accompanied by malignant progression and is summarized as abnormal proliferation of transition metals [10]. Program cell death (PCD), a cellular suicide program induced by multiple pathways including cuproptosis, ferroptosis, pyroptosis, necroptosis, apoptosis and paraptosis, all interact with copper to varying degrees [104]. Among these, cell death resulting from a series of copper-induced cascade reactions has been categorised as a separate form, cuproptosis [13]. Drugs targeting copper based on this mechanism are thought to have significant tumor therapeutic potential [105]. The interaction of copper with other forms of cell death complements the possible mechanisms of this class of drugs, but its bidirec-

tionality also warns of possible toxicological effects of the drugs.

4.1 | Cuproptosis

4.1.1 | Cuproptosis is a form of independent cell death with multiple contradictory pathways

Studies of disulfiram (DSF) and elesclomol (ES) have revealed that copper ion carriers can exert some antitumor effects in the tumor environment and are accompanied by an increase in oxidative stress products and the devastation DNA double strands [103, 106–109]. Thus, the conventional view is that the nature of cuproptosis is cell death due to oxidative stress caused by an increase in ROS. However, this notion was shattered by the findings of Tsvetkov et al. [110] Using a functional genomic approach, they first demonstrated that the inhibitor (PI) resistance state in the non-disturbed state is associated with a shift in mitochondrial energy metabolism. It was then found that ES promotes a distinct form of copper-dependent cell death in which ferredoxin 1 (FDX1) mediated synthesis of Fe-S clusters is inhibited by ES. Furthermore, the authors found that ES-induced cell death does not involve alterations in the activity of the apoptotic marker caspase 3, and additionally treatment with graded concentrations of inhibitors of ferroptosis, oxidative stress, and other inhibitors did not show a significant reduction in the outcome of ES-induced cell death, which is contrary to what has been previously recognized [111].

It is therefore clear that there are currently three main explanations for the pathway of cuproptosis: cell death is induced by enhancing oxidative stress in cells, inhibiting the ubiquitin-proteasome system (UPS), and targeting lipid acylated proteins in the tricarboxylic acid (TCA) cycle (Figure 2). The transition metal copper can generate intracellular ROS via the Fenton reaction, and the copper ion carriers DSF and ES can enhance this process to mediate cell death. Overloading of oxygen radicals such as hydroxyl radicals induced by carrier-Cu complexes achieves cuproptosis through mechanisms such as DNA damage, blocking the cell cycle, and regulating the p38 mitogen-activated protein kinase (P38MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) pathways of cell growth [109, 112, 113]. The UPS exists within cells and is responsible for keeping protein mass in equilibrium. Signals that disrupt this system may offer therapeutic promise by providing a pathway for cancer cell death [114, 115]. Copper ions can already put the UPS in an imbalanced state and induce cuproptosis through different mechanisms. Skrott et al. [116, 117] demonstrated that the DSF-Cu complex truncates signaling upstream of the proteasome system and inhibits ubiquitylated ATP synthase, and further found that the anticancer mechanism of DSF kills cells through the aggregation of copper-containing metabolites (CuET) to a subunit of the p97/VCP segregase adaptor (nuclear protein localization 4 [NPL4]) to kill cells. Chen et al. [107] study found that DSF-Cu was able to reduce tumor tissue proteasome activity and selectively inhibit UPS in tumor cells, but this process was also thought to be achieved through the apoptotic pathway. Key enzymes for lipoylation in mitochondria include dihydrolipoamide branched chain transacylase (DBT), glycine cleavage system H protein (GCSH), dihydrolipoamide S-acetyltransferase (DLST) and dihydrolipoamide acetyltransferase (DLAT), providing essential lipoylated proteins for the TCA cycle [118], and copper binds directly to and induces oligomerization of lipoylated DLAT. Downstream acute proteotoxic stress induced by damage to lipoylated proteins and Fe-S cluster proteins, the targets of cuproptosis, is the direct cause of cuproptosis [111, 119].

4.1.2 | Pan-cancer analysis of cuproptosis-related genes

Cuproptosis-related genes are integral in maintaining cellular homeostasis by modulating copper ion metabolism, which is critical for various biochemical pathways and cellular functions [120]. These genes regulate copper uptake, distribution, and storage, preventing copper-induced toxicity that can lead to oxidative stress and cellular damage [121]. In apoptosis, cuproptosis-related genes facilitate programmed cell death in response to copper ion accumulation, thereby controlling cell survival in a manner that can limit cancer progression [122, 123]. Additionally, these genes are involved in cell cycle regulation [119], where they influence key checkpoints and ensure that cells with damaged DNA or imbalanced copper levels do not proliferate uncontrollably. This multi-faceted role positions cuproptosis-related genes as potential therapeutic targets, particularly in cancers where copper metabolism is dysregulated [124].

Based on an extensive literature review, we have identified and summarized the classical cuproptosis-related genes along with the current characterization of their functional roles (Table 1) [120]. However, the specific functions and prognostic value of these genes across different cancer types remain unclear. Therefore, we conducted a pan-cancer analysis of these cuproptosis-related genes, covering multiple levels including the transcriptome, genome, and epigenome, and integrated survival data to explore their potential in cancer therapy (Figure 3). To perform this analysis, we retrieved the latest TPM expression matrix, somatic mutation data, copy number

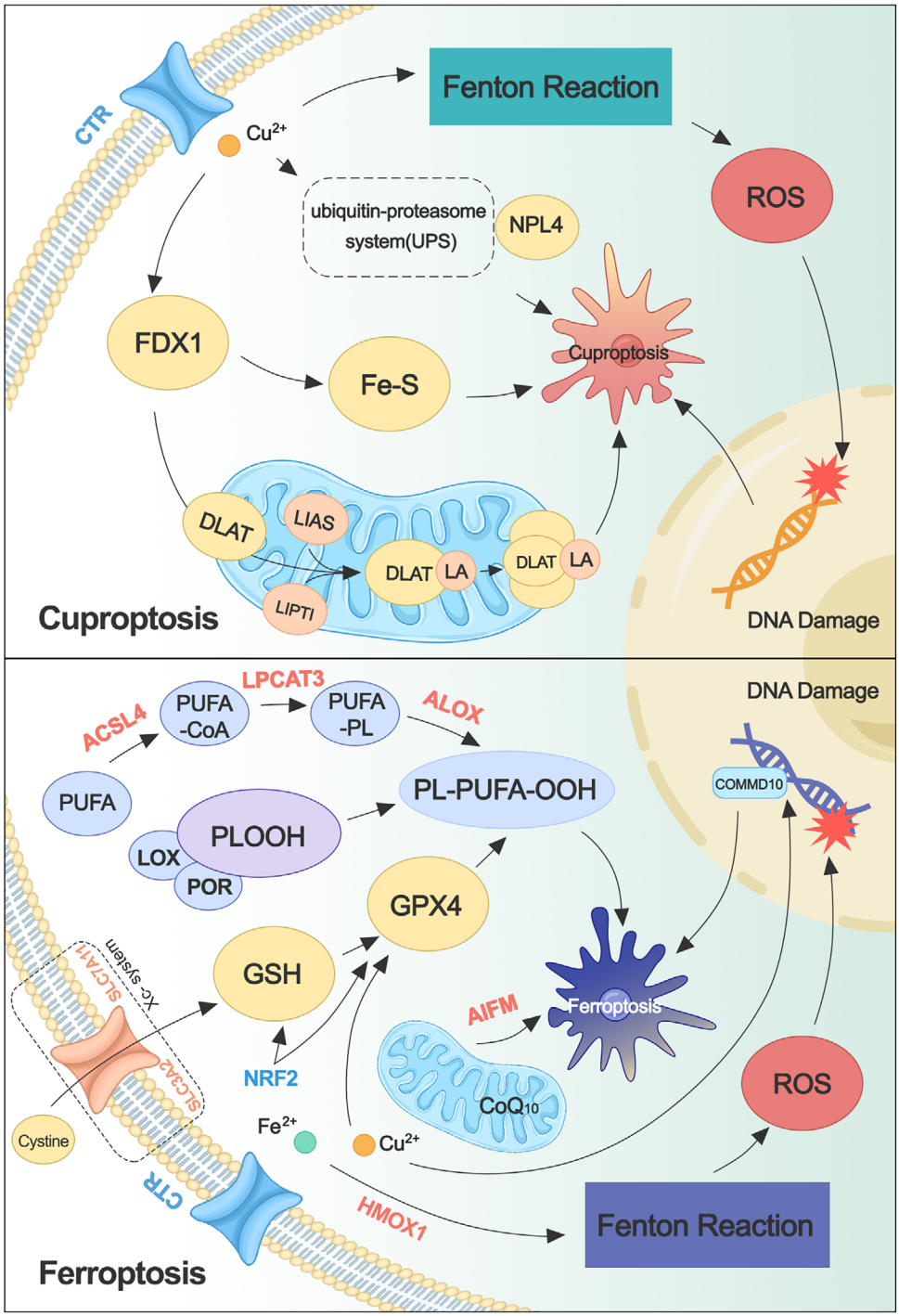

FIGURE 2 Copper landscape in cuproptosis and ferroptosis. The figure shows three pathways of cuproptosis: oxidative stress pathway, UPS pathway and lipid acylation pathway. In the oxidative stress pathway, copper generates ROS through the Fenton reaction, and the excess ROS exceeds the detoxification capacity of the cell, causing DNA damage and cuproptosis. Copper can activate NPL4 to inhibit ubiquitinated ATP synthase and impede the functioning of the proteasome system. FDX1, one of the upstream switches of protein lipidation, regulates the oligomerization of DLAT in which copper participates via the LA pathway, and also participates in the assembly of iron-sulfur clusters, Fe-S, to intervene in the transfer of the electron chain in both pathways causing cuproptosis. Abbreviations: ACSL4, acyl-coA synthetase long-chain family member 4; AIFM, apoptosis-inducing factor mitochondrial; ALOX, arachidonate lipoxygenase; CoA, coenzyme A; COMMD10, copper metabolism MURR1 domain 10; CTR, copper transporter; DLAT, dihydrolipoamide acetyltransferase; FDX1, ferredoxin 1; GPX4, glutathione peroxidase 4; GSH, glutathione; HMOX1, heme oxygenase 1; LA, linoleic acid; LIAS, lipoic acid synthetase; LIPT1, lipoyltransferase 1; LOX, lipoxygenase; LPCAT3, lysophosphatidylcholine acyltransferase 3; NPL4, nedd4-interacting protein 4; NRF2, nuclear factor erythroid 2-related factor 2; PL, phospholipid; PLOOH, phospholipid hydroperoxide; POR, P450 oxidoreductase; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species.

TABLE 1 Cuproptosis related genes.

Gene	Gene full name	Function	Impact on tumors
FDX1	Ferredoxin 1	Reduction of divalent copper ions; involved in electron transfer from NADPH to cytochrome P450	Bi-directionality
DLD	Dihydrolipoamide dehydrogenase	Constitutes the E3 component of the pyruvate dehydrogenase complex and is involved in the lipoic acid pathway	Bi-directionality
LIPT1	Lipoyltransferase 1	Involved in the lipoic acid pathway, transferring the lipoyl fraction to apolipoproteins	Promote
LIAS	Lipoic acid synthetase	Final step in the catalytic lipoic acid synthesis pathway	Inhibitory
DLAT	Dihydrolipoamide s-acetyltransferase	Synthesis of pyruvate dehydrogenase for protein lipoylation to obtain acetyl coenzyme A	Promote
DBT	Dihydrolipoamide branched chain transacylase E2	Synthesis of pyruvate dehydrogenase for protein lipoylation to obtain acetyl coenzyme A	Inhibitory
DLST	Dihydrolipoamide S-succinyltransferase	Synthesis of pyruvate dehydrogenase, which catalyzes the overall conversion of 2-oxoglutarate to succinyl coenzyme A and CO	Promote
GCSH	Glycine cleavage system protein H	Forms a glycine cleavage system that transfers the methylamine moiety of glycine from P proteins to T proteins and regulates amino acid metabolism	Promote
PDHA1	Pyruvate dehydrogenase e1 subunit alpha 1	Catalyzes the overall conversion of pyruvate to acetyl coenzyme A and CO and regulates sugar and lipid metabolism	Inhibitory
PDHB	Pyruvate dehydrogenase e1 subunit beta	Catalyzes the overall conversion of pyruvate to acetyl coenzyme A and CO and regulates sugar and lipid metabolism	Inhibitory
CDKN2A	Cyclin dependent kinase inhibitor 2A	Blocking the cell cycle at G1 and G2 stages	Promote
SLC31A1	Solute carrier family 31-member 1	Dominant copper intake	Promote
ATP7A	ATPase copper transporting alpha	Involved in copper excretion (extrahepatic tissues)	Inhibitory
ATP7B	ATPase copper transporting beta	Involved in copper excretion (mainly in intrahepatic tissues)	Inhibitory

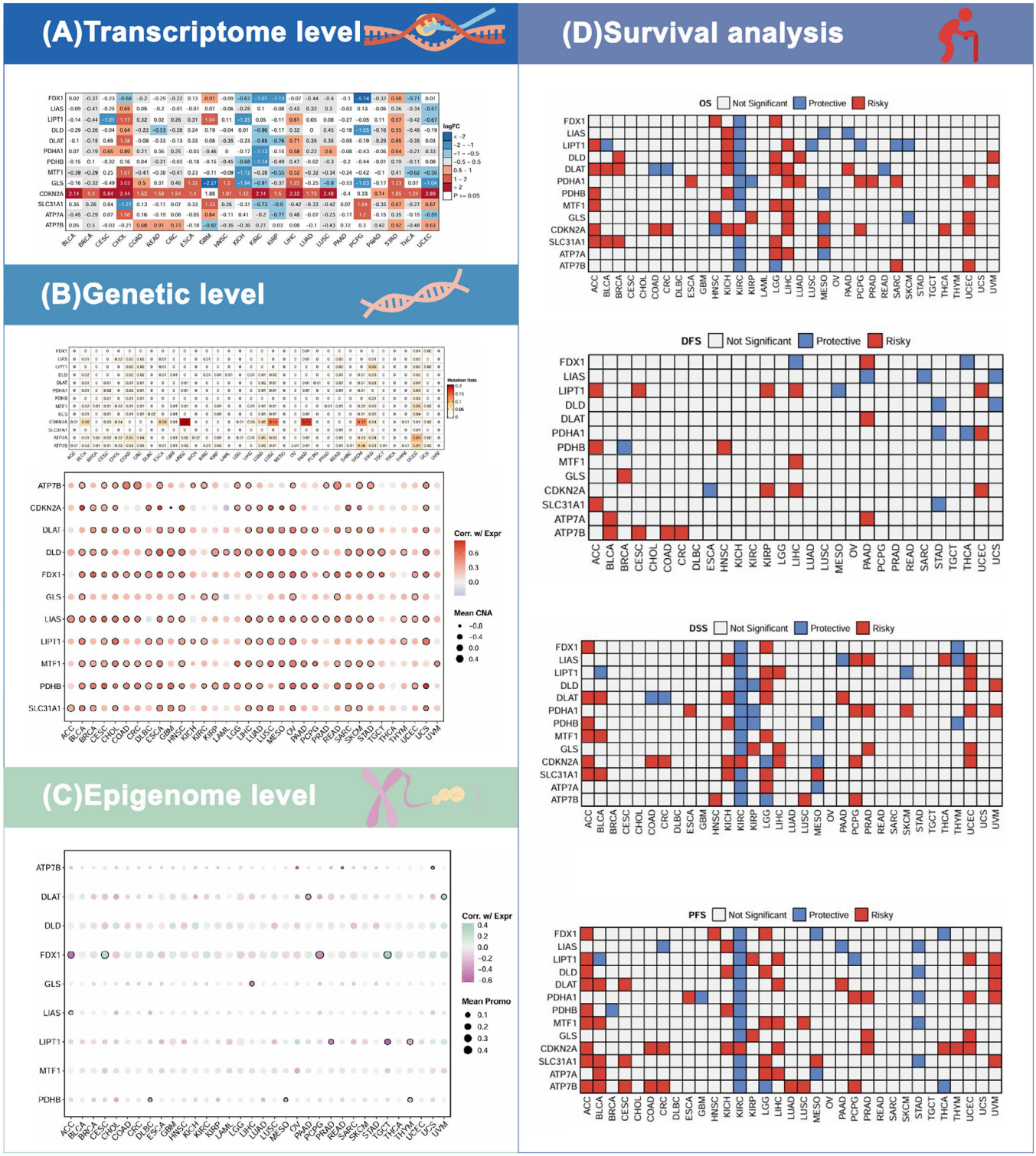

FIGURE 3 Results of pan-cancer analysis. (A) Results of normal and tumor tissue differential analysis of cuproptosis-related genes in pan-cancer transcriptome. (B) Proportion of gene mutations and CNAs of cuproptosis-related genes in pan-cancer genome. (C) Promoter methylation of cuproptosis-related genes in pan-cancer epigenome. (D) Survival analysis of cuproptosis-related genes in pan-cancer. Abbreviations: ACC, adrenocortical carcinoma; ATP7A, ATPase copper transporting alpha; ATP7B, ATPase copper transporting beta; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CDKN2A, cyclin dependent kinase inhibitor 2A; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; CRC, colorectal carcinoma; DFS, disease-free survival; DLAT, dihydrolipoamide s-acetyltransferase; DLBC, diffuse large B-cell lymphoma; DLD, dihydrolipoamide dehydrogenase; DSS, disease-specific survival; ESCA, esophageal carcinoma; FDX1, ferredoxin 1; GBM, glioblastoma multiforme; GLS, glutaminase; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, low-grade glioma; LIAS, lipoic acid synthetase; LIHC, liver hepatocellular carcinoma; LIPT1,

variation (CNV) data, 450K methylation profiles, and survival data from the TCGA database (https://portal.gdc.cancer.gov/) via the TCGAbiolinks R package (v2.3.4) using the GDCquery, GDCdownload, and GDCprepare functions. Gene expression values in the TPM matrix were log-transformed as log2(TPM + 1) for downstream analysis. Differential expression analysis was conducted with the limma package. Mutation frequencies were calculated as the ratio of each gene's mutation count within individual tumors to the total number of samples. Pearson correlation coefficients were employed to examine the association between CNV values and gene expression levels. Methylation probes within the TSS-island region were selected to represent the promoter region, and Pearson correlation was computed between the beta values of these probes and gene expression levels. Cox proportional hazards regression was applied to assess the prognostic significance of each gene. Additionally, we developed the SLTCGA R package, accessible at https://github.com/Zaoqu-Liu/SLTCGA, to streamline gene exploration within the TCGA database.

The results indicate that cyclin-dependent kinase inhibitor 2A (*CDKN2A*) exhibits high cancer specificity at both the transcriptomic and genomic levels, with significantly elevated expression and mutation rates across multiple cancer types, such as breast cancer (BRCA), lung adenocarcinoma (LUAD), and skin cutaneous melanoma (SKCM) (Figure 3A-B). This distinct profile suggests that *CDKN2A* may play a crucial role in copper-induced cell death pathways in cancer, potentially acting as a key regulator of cellular processes under oxidative stress. The *CDKN2A* gene encodes two proteins, $p16^{INK4a}$ and $p14^{ARF}$, which play an important role in cell cycle regulation and arrest the cell cycle in G1 phase [125]. Oxidative stress induced by copper ions activates the *CDKN2A* gene to regulate cell cycle and apoptosis via the p53 pathway [126, 127]. The epigenetic analysis further highlights the unique role of FDX1 in various cancer types. FDX1 shows significant variability in promoter methylation across multiple cancers, with a notable negative correlation between methylation levels and gene expression, particularly in cancers such as BRCA and LUAD (Figure 3C-D). This suggests that epigenetic regulation of FDX1 may play a crucial role in modulating its activity in copper ion reduction and other cellular processes, such as steroidogenesis and iron-sulfur cluster formation. During cuproptosis, FDX1 acts as an upstream regulatory switch in the proteolipid acylation pathway, facilitating copper's involvement in the lipoic acid (LA) pathway and enabling the cell death process [128–130]. In addition, genes such as SLC31A1 and ATP7A/B are the upstream channels of cuproptosis due to their direct involvement in copper metabolism [131], and we consider such genes to be direct cuproptosis genes; whereas, DLD, DLAT, PDHA1, PDHB, etc. [13, 132], are more related to the tricarboxylic acid cycle and are downstream channels of cuproptosis by causing metabolic disorders that lead to cell death, and are therefore considered to be indirect cuproptosis genes.

The results of survival analysis suggested that copper-related genes have an overall impact on survival, but with notable variability depending on the type of cancer. Metabolism-dependent cancers, such as liver hepatocellular carcinoma (LIHC) and kidney renal clear cell carcinoma (KIRC), are more likely to show a strong correlation with cuproptosis.

4.1.3 | The effects of cuproptosis on tumors are two-sided

Cuproptosis and tumors exhibit a clear bidirectional relationship. On the one hand, high copper concentrations accompany the development of some cancer types and are even associated with poorer progression and prognosis. Serum copper levels have been found to be significantly elevated in a variety of cancers, including oral cancer [133], bladder cancer [134], female genital malignancies [135, 136], prostate cancer [137, 138], breast cancer [139, 140], stomach cancer [141], colon cancer [138], pancreatic cancer [142], gallbladder cancer [143], lung cancer [144], and thyroid cancer [145, 146]. However, this correlation cannot be considered equivalent to a pro-tumorigenic effect; the simultaneous increase in copper concentration and tumor cells may imply a higher demand for copper for the growth of cancer cells, and again does not exclude the generation of antagonistic mechanisms by the organism in response to the onset of tumor cells, in which case the copper implies anti-tumorigenic efficacy. In contrast, studies of copper ion carriers have suggested an

lipoyltransferase 1; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; MTF1, metal response element binding transcription factor 1; OS, overall survival; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PDHA1, pyruvate dehydrogenase e1 subunit alpha 1; PDHB, pyruvate dehydrogenase e1 subunit beta; PFS, progression-free survival; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; SLC31A1, solute carrier family 31 member 1; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

anti-tumor aspect of copper. Paclitaxel in combination with ES significantly increased progression-free survival in melanoma [108] and DSF-copper complexes proved to be effective in inhibiting breast cancer MDA-MB-231 cells with xenografted tumors [107]. Mechanism of cuproptosis combined with studies of cancer genes may refine copper therapy Moison et al. [147] obtained an optimized copper ion carrier, UM4118, which promotes cuproptosis when co-existing with down-regulation of the mitochondrial iron-sulfur cluster transport protein ABCB7 in the presence of a mutation in *SF3B1*. It is suggested that *SF3B1* can be used as a reliable biomarker for copper therapy [147].

During tumorigenesis and development, selective enrichment of inflammatory factors occurs in the local environment, so targeting the tumor immune microenvironment has become one of the focuses of anti-tumor therapy. It is interesting to note that copper ions can also cause non-unitary effect on tumors in the body through the immune pathway, but this part of the mechanism is less well understood. Liao et al. [148] revealed the presence of IL-17-STEAP4-XIAP axis, and the action of IL-17 on STEAP proteins induces a rise in intracellular copper content, and the signal of elevated copper concentration ultimately activates the XIAP and NFκB pathways to promote colon carcinogenesis. In addition, cuproptosis cells are capable of triggering sterile inflammation using high-mobility group box 1 (HMGB1) as a mediator, and the mechanism is mainly based on the activation of AMP-activated protein kinase (AMPK) by intracellular high-copper depletion of ATP as an upstream pathway. This pathway promotes cuproptosis in pancreatic and hepatocellular carcinoma cells. Future copper targeted therapies might be used to modify the composition of the tumor microenvironment.

4.2 | Copper-associated cell death

Copper has a high while frequency and presence in all types of cell death pathways, especially ferroptosis. The pathways of ferroptosis include an exogenous pathway that inhibits the amino acid reverse transporter System Xc- and an endogenous pathway that inhibits glutathione peroxidase 4 (GPX4) [149, 150] (Figure 2). The intersection of ferroptosis and cuproptosis is centered on GPX4 and hypoxia-inducible factor 1 subunit α (*HIF1α*). The anti-ferroptosis effect of *GPX4* is mainly mediated by the TAX1BP1 receptor [151]. Copper was found to bind to cysteine residues of *GPX4*, causing autophagic degradation of *GPX4* proteins and reducing the ability to detoxify oxidized lipids, thus enhancing susceptibility to ferroptosis [151]. It is worth noting that the ubiquitin-proteasome pathway also mediates degradation of *GPX4* proteins, and in combination with the pathway of ferroptosis, which may be a new crossover point [152]. *HIF1A* is thought to be a pro-survival factor for ferroptosis [153]. Low expression of copper metabolism MURR1 domain 10 (COMMD10) induced cuproptosis accumulation, impaired its binding to and inhibited ubiquitin degradation, and ultimately caused the up-regulation of *HIF1α* downstream of the fatty acid binding protein 3 (*FABP3*) and fatty acid binding protein 7 (*FABP7*) promoted lipid storage and inhibited ferroptosis in hepatocellular Carcinoma(HCC) cells [154]. In recent clinical practice, photodynamic therapy based on microwave-induced cupro-cysteamine (Cu-Cy) nanoparticles depletes GPX4 and induces ferroptosis on colorectal cancer cells [155]. Given that cuproptosis also acts as an important regulator in ferroptosis, focusing on ferroptosis-associated genes will help us to further analyze the association between cuproptosis and ferroptosis as well as the role it plays in cancer. We therefore summarize the ferroptosis-related genes and their basic roles (Table 2).

In addition to ferroptosis, PCD including apoptosis, paraptosis, necroptosis, pyroptosis also involves copper. The endogenous pathway of apoptosis can be stimulated by mitochondrial damage signaling to cytochrome c or apoptosis inducing factor mitochondria associated 1/apoptosis inducing factor (*AIFM1/AIF*) release, a process controlled by the B-cell lymphoma (*BCL*) family of proteins that ultimately causes downstream cascade activation of caspases [104]. A study of rat testes damaged by environmental exposure to copper nanoparticles showed that the presence of copper upregulated the expression of the apoptosis-associated protein Bax, a member of the BCL family, and that this change was mediated through the Akt/mTOR signaling pathway [156]. Taking advantage of the apoptotic properties of PC12, Kawakami et al. [157] treated with copper and found that Bax may alter mitochondrial membrane potential through two pathways, the caspase9-dependent cytochrome c pathway and the caspase-independent AIF pathway. Unlike apoptosis, Paraptosis is not dependent on caspases and is usually characterized by swelling of organelles represented by the endoplasmic reticulum [158]. Inhibition of the UPS is one of the major pathways of cuproptosis, and the stacking of toxic proteins causes cellular stress that triggers paraptosis, thereby cross talking copper with paraptosis [159]. The mechanism by which copper promotes apoptosis has now been further demonstrated in cancer therapy. Guo et al. [160] designed a Ca^{2+}/Cu^{2+} dual-ion "nano trap" through mitochondrial calcium overload, dithiocarbamate, and the generation of toxic substances such as free radicals, inducing apoptosis and parapoptosis simultaneously. However, the relationship between copper and pan-apoptosis (PANoptosis) is not all promotive [161], and differentiated and quiescent cells are able to

TABLE 2 Ferroptosis related genes.

Gene	Gene full name	Function	Impact on tumors
ACSL4	Acyl-CoA synthetase long chain family member 4	Regulates lipid biosynthesis and fatty acid degradation by converting free long-chain fatty acids into fatty acyl coenzyme A esters	Bi-directionality
GPX4	Glutathione peroxidase 4	Catalyzed reduction of hydrogen peroxide, organic hydroperoxides and lipid hydroperoxides	Promote
NRF2	NFE2 like bZIP transcription factor 2	Regulation of the expression of antioxidant proteins involved in inflammatory and injury responses	Promote
SLC7A11	Solute carrier family7 member 11	Involved in the formation of System Xc-	Promote
TP53	Tumor protein p53	Induce cell cycle arrest, apoptosis, senescence, DNA repair or metabolic changes	Inhibitory
HMOX1	Heme oxygenase 1	Cleaves hemoglobin to form bilirubin, which produces antioxidants	Inhibitory

counteract the apoptotic effect, such as neuronal cells and glucocorticoid-dependent cells [162]. Designing copper to counteract apoptotic effects on different cells may allow for more precise cancer targeting.

Necroptosis leads to cell lysis and content egress, a process induced primarily by death receptors on the cell membrane. The microscopic manifestation is the phosphorylation of mixed lineage kinase domain-like (MLKL) proteins by the necrosome protein signaling complex assembled by receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and receptor-interacting serine/threonine-protein kinase 3(RIPK3), which initiates necroptosis [163, 164]. A study of ω-3 demonstrated its mitigating effect on copper neurotoxicity-attenuating interleukin-1 beta (IL-1β) release and mitogen-activated protein kinase (MAPK) activation-flanking the theory that copper generates cross-talk with Necroptosis via the MAPK/NF-kB/MLKL axis [165]. However, direct evidence for further cross and causal associations is lacking [105, 166]. Pyroptosis is an immune cell death mediated by gasdermin, triggered by the activation of classical or non-classical inflammasomes by caspas-1 or caspas-4/5/11 [167, 168]. Copper was shown to primarily target caspase-1 to induce pyroptosis. copper treatment of chicken hepatocytes was found to generate reactive oxygen species ROS that caused elevated expression of pyroptosis-related genes (Caspase-1, IL-1β, IL-18, and NLRP3) and caspase-1 [169]. Excess copper was found to induce endoplasmic reticulum swelling and elevated caspase-1 signaling, in contrast, 4-phenylbutyric acid and MKC-3946 alleviated the response, suggesting that the endoplasmic reticulum pathway is triggered by copper mainly through the IRE1α-XBP1 pathway [170].

Cell death is the bridge between copper and tumors, and by controlling cell death, copper could show a bidirectional effect on tumors. For example, in nasopharyngeal carcinoma cells, DSF-Cu occurs through the ROS/MAPK and p53-mediated ferroptosis pathway. In recent clinical practice, photodynamic therapy based on microwave-induced Cu-Cy nanoparticles depletes *GPX4* and induces ferroptosis on colorectal cancer cells [155]. Examples of the use of cuproptosis therapy to enable therapeutic exploration of cancer through the mechanism of other cell death pathways are relatively scarce, although it is feasible in principle. This may be due to the fact that the role played by copper has been overshadowed, resulting in less attention being paid to it.

5 | COPPER IS INVOLVED IN ALL ASPECTS OF TUMOR METASTASIS

Tumor metastasis encompasses three stages: dissemination, dormancy and colonization [171]. The increase in clonality of tumor cells at the primary site, driving the regulation of the tumor immune microenvironment and enhancing the viability of cancer cells are all adaptations that tumor cells make to expand their sphere of influence within the body. In this regard, the involvement of copper in tumor cell growth and proliferation, energy metabolism, and angiogenesis has a considerable impact on the metastatic process (Figure 4, Table 3).

5.1 | Cell growth and proliferation

The receptor tyrosine kinase (RTK) signaling cascade regulates cellular responses to extracellular stimuli such as chemokines and directs cell proliferation [172]. Consequently, RTKs are often overactivated in cancer. Copper binding to upstream RTK causes activation of apoptosis tyrosine kinase (ATK) and extracellular

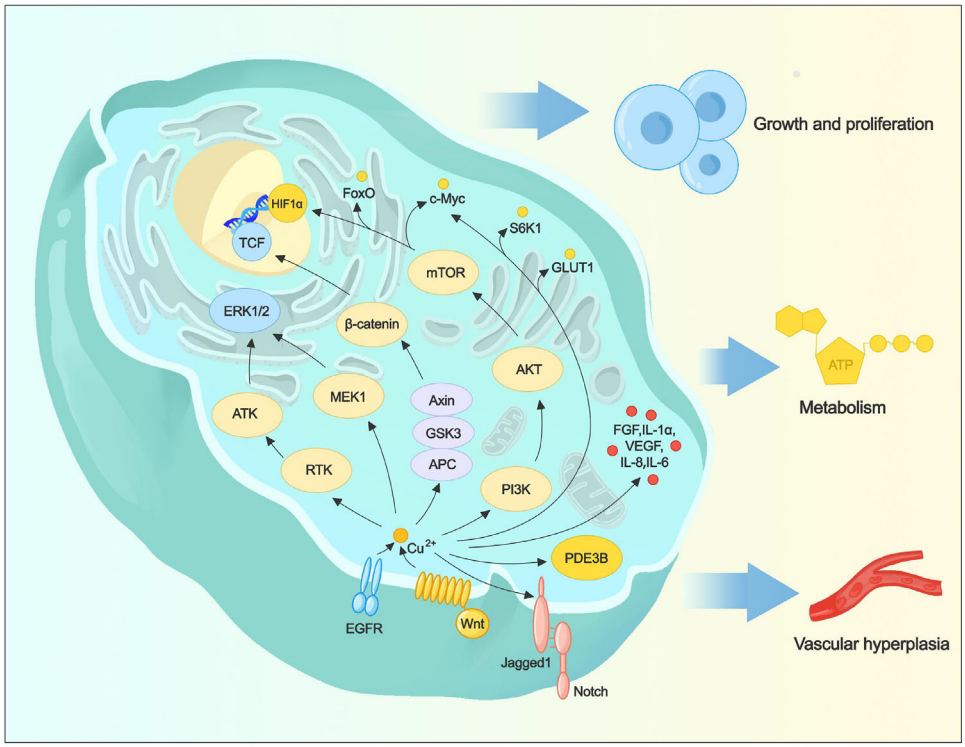

FIGURE 4 Copper in tumor metastasis. In tumor metastasis, copper acts at three main levels: growth and proliferation, energy metabolism and vascular proliferation. Pathways related to growth and proliferation include RTK, MAPK, ULK, Notch and Wnt pathways. Pathways related to energy metabolism include the PI3K-Akt-mTOR pathway, and lipid and glucose metabolism pathways. Vascular proliferation is achieved through direct or indirect production of pro-angiogenic factors by copper.
Abbreviations: AKT, protein linase B; APC, adenomatous polyposis coli; ATK, AKT/protein kinase B; c-Myc, cellular myelocytomatosis viral oncogene homolog; EGFR, epidermal growth factor receptor; ERK1/2, extracellular signal-regulated kinase 1/2; FGF, fibroblast growth factor; FoxO, forkhead box O; GLUT1, glucose transporter 1; GSK3, glycogen synthase kinase 3; *HIF1α*, hypoxia-inducible factor 1 alpha; IL-1α, interleukin 1 alpha; IL-6, interleukin 6; IL-8, interleukin 8; mTOR, mammalian target of rapamycin; PDE38, phosphodiesterase 38; PI3K, phosphoinositide 3-kinase; RTK, receptor tyrosine kinase; S6K1, S6 Kinase 1; TCF, T-cell factor; VEGF, vascular endothelial growth factor; Wnt, wingless/int1.

signal-regulated protein kinase (ERK) [173], which crosses over with the MAPK pathway. The MAPK pathway is activated sequentially by tertiary kinases and is usually located downstream of growth factor receptors [174]. There are four branches of this pathway, with ERK being the significant one and is in charge of cell growth and differentiation [175]. Copper ions act before ERK binds to mitogen-activated protein kinase 1 (MEK1), producing pro-tumor growth activity [176, 177]. Knockdown of CTR1 levels and mutations in MEK1 have been found to provide inhibitory signaling to *BRAF*, suggesting that cancers exhibiting *BRAF* properties hold promise for targeted copper therapy [178]. The Notch pathway is usually regarded as a tumor suppressor widely involved in the development of malignant tumors [179]. But is now recognized as having a bidirectional regulatory role in cancer [180], which is a development of the traditional view of cancer inhibition [179]. The Notch receptor has five ligand counterparts in the human body, Jagged 1, Jagged 2, Delta 1, Delta 3, and Delta4. It has been observed that physiological concentrations of copper ions induce hydrolysis of the adhesion molecule E-cadherin and the Notch ligand Jagged1, suggesting that copper may inhibit the Notch pathway by shedding Jagged1 [181]. The Wnt signaling pathway is mainly mediated and realized by β-catenin, and its abnormality mainly interferes with the pluripotent differentiation of cancer stem cells (CSCs), which is thought to be strongly associated with cancer recurrence [182–184]. The copper ion carrier, DSF, has been found to inhibit cancer cell growth and metastasis by modulating the Wnt/β-catenin signaling pathway and stress response [185]. RTK, MAPK, ULK, Notch, Wnt and other pathways are intertwined, constituting a complex network of proteins [186]. Copper ions affect the growth process of cancer cells by acting on key protein kinases, so it is even more important to pay attention to discerning the role of copper in the study of copper-related therapeutic drugs.

TABLE 3 Genes associated with copper metabolism and tumor growth and metastasis.

Gene	Signal pathways involved	Relationship with copper metabolism	Impact on tumors
CK1γ, hoxb5b	Wnt/β-catenin signaling pathway	Down-regulation of the pathway through oxidative stress pathways such as COX17 and SOD1	Inhibitory
FoxO1a	PI3K/AKT signaling pathway	Copper exerts an insulin mimetic effect to activate the pathway	Promote
RTK	RTK signaling pathway	Copper binds to upstream RTK to activate ATK and ERK	Promote
MEK1	MAPK signaling pathway	Copper binding to upstream RTK activates ATK and ERK; Cu-MEK1 interaction enhances phosphorylation of ERK1/2 by MEK1	Promote
SMAD2	TGF-β signaling pathway	Copper downgraded the occurrence of this pathway	Inhibitory
NF-κB	NF-κB signaling pathway	Copper activates the transcription factor NF-κB with the aid of oxidative stress	Bi-directionality
Jagged 1	Notch signaling pathway	Copper inhibits the Notch pathway by shedding Jagged1 hydrolysis	Promote
ULK1	Autophagy signaling pathway	Activation of cellular autophagy pathway upon copper overload	Bi-directionality

Abbreviations: CK1γ, casein kinase 1 gamma; AKT, protein kinase B; ATK, AKT/protein kinase B; COX17, cytochrome coxidase assembly protein 17; ERK, extracellular signal-regulated kinase; FoxO1a, forkhead box O1A; hoxb5b, homeobox B5B; MAPK, mitogen-activated protein kinase; MEK1, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K, phosphoinositide 3-kinase; RTK, receptor tyrosine kinase; RTK, receptor tyrosine kinase; SMAD2, SMAD family member 2; SOD1, superoxide dismutase 1; TGF-β, transforming growth factor beta; ULK1, Unc-51 like autophagy activating kinase 1; Wnt, wingless/int1.

5.2 | Metabolism pathway

Activation of the PI3K-Akt-mTOR pathway dominates cancer cell metabolism, which is partly realized by insulin activation of mTORC1, and additionally promotes cell growth when insulin-like growth factor (IGF) is involved [187]. Among them, the downstream transcription factors of mTOR include molecules such as HIF1α, c-Myc, and FoxO in response to various nutrients. Copper exposure can cause FoxO1a to be activated, at which point it exerts an insulin-like effect, stimulating cancer cells to take up more glucose to accumulate energy for metastasis [188–190]. In addition to the aforementioned cell growth-related pathways, the metabolism of tumor cells themselves is also an important factor affecting migration, including lipid metabolism and glucose metabolism. Cuproptosis is recognized as a metabolic cell death due to its close relationship to energy metabolism pathways [119]. Therefore, all energy metabolism-related pathways may have copper involvement, which is also well demonstrated experimentally. From previous discussions, we know that not only cuproptosis is involved in the process of lipid acylation, but copper is also involved in regulating the lipid metabolic content associated with ferroptosis. Therefore, copper can serve as a regulator of lipid metabolism. In a study of the Wuhan-Zhuhai cohort, researchers explained the association between copper exposure and the development of dyslipidemia and suggested that this association is mediated in part by the involvement of systemic inflammation [191]. Using a mouse model of inherited copper dysregulation, Krishnamoorthy et al. [192] found that copper alters the activity of the enzyme phosphodiesterase PDE3B, with a key action site at the cysteine residue, a process that exemplifies copper's regulation of lipid metabolism at the molecular level. In addition to this, it is interesting to note that that copper also inhibits glycolysis by inhibiting S6K1, c-Myc, and GLUT1, a phenomenon that is consistent with the insulin-like effects of copper [193].

5.3 | Angiogenesis

In addition to the above factors affecting the value-added migration of cancer cells, another crucial factor influencing tumor migration is angiogenesis. As early as 1980, McAuslan et al. [194] suggested that copper ions could induce the phenomenon of endothelial cell migration, which implies that copper has pro-angiogenic properties. Using siRNA to silence CTR1 and block copper entry into cells, a significant reduction in proliferation, migration and angiogenesis was observed [195]. In addition, copper can regulate angiogenesis-related factors, including fibroblast growth factor (FGF), inflammatory cytokine (IL-1α), vascular endothelial growth factor (VEGF), IL-8 and IL-6 [40, 196–198]. Many studies have shown that copper upregulates hypoxia-inducible factor 1 (HIF1), a process that is CCS-dependent [40] and regulates oxygen binding to hemoglobin. Notably, VEGF, a target gene of HIF-1α, can

be indirectly affected by copper to promote angiogenesis [199].

6 | Exploring the clinical application of copper

A summary of copper metabolism suggests a richly argued cancer-related mechanism in oxidative stress, cell death pathways, and tumor metastatic pathways, although this effect is two-sided. Thus, copper has a predictable and valuable future in clinical laboratory as well as in tumor therapy.

6.1 | Clinical assessment of copper levels in humans

Cancer cells exhibit an abnormally high demand for growth, including factors such as oxidative energy supply, metabolic levels, proteasome, and angiogenesis, and all these physiological processes have been shown to involve copper. As of now, there is considerable experimental data demonstrating a strong correlation between tumors and high copper levels, which means that effective testing can increase the cancer detection rate.

The most common indicator, serum CP (sCP), a multicopper oxidase, can carry up to six copper atoms and reflects copper status in plasma due to containing over 95% of its copper [200]. The reference value for copper-cyanin varies when using different methods, ranging from 200 to 500 mg/L by immunoscattering turbidimetry, 300 to 650 mg/L by immunodiffusion for 12 years and under, and 150 to 600 mg/L for adults. The association of sCP with cancer has been confirmed in several experiments, suggesting that it may be used as a reference indicator for certain cancers. Ionizing radiation was found to cause an increase in downstream CP transcription, and ferroptosis in hepatocellular carcinoma cells was thus inhibited, confirming the positive regulation of CP on cancer cells [154]. Moreover, lncRNA cuprocyanin (NRCP) was highly expressed in ovarian tumors, and was thought to intervene in glycolysis and thus affect cancer cell proliferation [201].

Twenty-four hours urinary copper, hepatic copper, fecal copper, and non-CP-bound copper can also be used as indicators to assess copper status [202, 203]. However, despite the ability of non-CP-bound copper to reflect changes in status in both copper-poor and copper-rich individuals, there is still a lack of validated measurements. In addition, fecal copper testing is mainly used in clinical studies and rarely appears in practical applications.

Copper homeostasis is based on the regulation of copper enzymes. When the intracellular copper level is low, the activity of copper-transferring enzymes tends to increase while the activity of copper-transferring enzymes decreases, so the measurement of copper enzyme activity can more indirectly and accurately reflect the copper status. Another indirect indicator of copper status is blood cell count. Some scholars believe that copper-poor status is accompanied by a decrease in white blood cell count, and the rest of the blood cell indexes may change, but there is a lack of experimental data on the index, which is still controversial [200, 204]. Unfortunately, there is a lack of research on the above indicators as cancer observation indicators, and their practical application reference value is unknown.

6.2 | Copper-based cancer therapeutics

Drugs such as copper ion carriers were used once in the treatment of Wilson's disease and Menkes' disease. The reuse from older drugs for copper metabolism-related diseases may offer new hope in the quest for copper-associated cancers (Table 4). Is copper our ally or enemy? In fact, both copper therapies have shown promising results. Copper chelator, which work by elevating intracellular copper ions, and copper inhibitors, which work by lowering copper levels, are two diametrically opposed medications that can take different paths to the other side of the cancer battle.

6.2.1 | Copper chelator

Copper chelators are a class of anticancer drugs, including copper ion carriers, copper-containing compounds, and copper nanoparticles, that directly or indirectly induce cancer cell destruction by increasing intracellular copper levels.

Copper ion carriers that are commonly used as investigational agents in clinical settings include DSF and ES, among others. The drug DSF, which has worked in the clinic as a release agent for alcohol dependence, is currently attracting renewed attention because of its antitumor activity [205, 206]. The traditional DSF antitumor mechanism derived from aldehyde dehydrogenase (ALDH) has been challenged [117, 207]. Indeed, DSF is rich in anti-tumor pathways, including induction of oxidative stress, inhibition of 20S proteasome-induced cuproptosis and ferroptosis, triggering of autophagy, activation of the MAPK pathway, and inhibition of nuclear factor NFκB [107, 112, 208–210], dependent on the binding of their metabolites to Cu for realization. There have been many explorations of applying DSF to treat cancer. Mice with xenograft breast cancer can be significantly inhibited by

TABLE 4 Advances in copper-based classical clinical drugs.

Drug	Intervention/treatment	Mechanism	Type of cancer	Phase
DSF	DSF and cisplatin	Eradication of NTERA-2 model globules, inhibition of ALDH activity, and reconstitution of tumor sensitivity to cisplatin	Germ Cell Tumor	Phase II
	DSF and cisplatin	Enhancing tumor sensitivity to cisplatin using the antitumor activity of DSF	Advanced Gastric Cancer	Not Applicable
	DSF	Supplemental copper levels cause ROS accumulation	Breast Neoplasm Female Metastatic Breast Cancer	Phase II
	DSF; Copper Gluconate; Temozolomide; Surgery; Radiation	DSF shows multiple anti-tumor mechanisms, including upregulation of ROS levels, P-gp dysfunction, targeting of CSCs, inhibition of EMT, and modulation of the tumor immune microenvironment, which contributes to preoperative suppression of the tumor niche	Glioblastoma Multiforme	Phase I Phase II
	DSF; Copper gluconate; Temozolomide		Glioblastoma Glioblastoma Multiforme	Phase II
	DSF/Copper; Temozolomide		Recurrent Glioblastoma	Phase II
	DSF; Metformin		Glioblastoma	Early Phase I
	CUSP9v3 treatment protocol (Temozolomide; Aprepitant; Minocycline; DSF; Celecoxib; Sertraline; Captopril; Itraconazole; Ritonavir; Auranofin)		Glioblastoma	Phase I Phase II
	DSF; Copper Gluconate; Doxil	ALDH is involved in the development of resistance to chemotherapeutic drugs (e.g. liposomal doxorubicin), and DSF targets and inhibits ALDH, an effect that is enhanced by Cu	Relapsed Sarcomas	Phase I
	DSF and chelated zinc	Melanoma cell lines at different stages are sensitive to in vitro DSF treatment; the Cu complex is slightly more active against melanoma and is thought to be the active agent in DSF-induced toxicity. The redox transformation of DSF is specific to Cu (II).	Melanoma	Phase II
	DSF		Stage IV Melanoma	Phase I Phase II
	DSF; Arsenic trioxide		Metastatic Melanoma	Phase I
	Copper; DSF; Copper gluconate	DSF demethylates known highly methylated tumor suppressor genes (e.g., APC and RARß) in PCa cell lines, inhibiting PCa cell growth in vitro and in vivo.	Prostate Cancer	Phase I
	DSF		Prostate Cancer	Not Applicable
	DSF; Copper Gluconate	DSF exerts antitumor activity through upregulation of ROS levels, P-gp dysfunction, targeting of CSCs, EMT inhibition, and TME regulation.	Cancer (Refractory Solid Tumors Involving the Liver)	Phase I
	DSF; Gemcitabine Hydrochloride	DSF can trigger the death program of pancreatic cancer cells through cGAS-STING, PI3K/mTOR/p70S6K and other signaling pathways	Metastatic Pancreatic Adenocarcinoma Refractory Malignant Solid Neoplasm Stage IV Pancreatic Cancer AJCC v8	Phase I

(Continues)

TABLE 4 (Continued)

Drug	Intervention/treatment	Mechanism	Type of cancer	Phase
	Abraxane/Gemzar Protocol Plus DSF/Copper Gluconate; FOLFIRINOX regimen Plus DSF/Copper Gluconate; Single-agent Gemzar regimen Plus DSF/Copper Gluconate		Metastatic Pancreatic Cancer	Phase II
	Canagliflozin and Gemcitabine; Gemcitabine		Pancreatic Cancer	Not Applicable
	DSF; Copper Gluconate	Activation of the JNK pathway, exogenous and endogenous apoptotic pathways	Multiple Myeloma	Phase I
	Chemotherapy ± DSF	DSF inhibits neovascularization in tumors	Non-small Cell Lung Cancer	Phase II Phase III
ES	ES Sodium	ES, a mitochondrial stress inducer dependent on copper ion transport to exert cytotoxicity, exhibits strong inhibition of cancer stem cells, platinum-resistant cancer cells, proteasome inhibitors, molecularly targeted drugs, and glycolysis.	Acute Myeloid Leukemia	Phase I
	ES Sodium; Paclitaxel		Fallopian Tube Clear Cell Adenocarcinoma, etc.	Phase II
	ES Sodium		Metastatic Solid Tumors	Phase I
	ES; Paclitaxel		Neoplasms	Phase I
	ES		Soft Tissue Sarcoma	Phase II
	ES; Paclitaxel		Melanoma	Phase III
	ES; Paclitaxel		Melanoma	Phase I Phase II
	Paclitaxel; Carboplatin; ES		Stage IIIB Non-Small Cell Lung Cancer Stage IV Non-Small Cell Lung Cancer	Phase I Phase II
	ES Sodium; Docetaxel		Prostate Cancer	Phase I
Clioquinol	Clioquinol	Clioquinol is a copper-zinc ion carrier, proteasome inhibitor, anti-angiogenic agent, and inhibitor of key signaling pathways, inhibiting growth activity and cytotoxicity in cancer cells.	Acute Myeloid Leukemia; Acute Lymphocytic Leukemia; Chronic Lymphocytic Leukemia; Myelodysplasia; Lymphoma, Non-Hodgkin; Hodgkin's Lymphoma; Multiple Myeloma	Phase I

(Continues)

TABLE 4 (Continued)

Drug	Intervention/treatment	Mechanism	Type of cancer	Phase
TM	TM; Capecitabine; Pembrolizumab	TM inhibits tumor cell growth by inhibiting copper-dependent enzymes required for tumor cell proliferation, inducing tumor cell necrosis or loss-of-nest apoptosis, increasing oxidative stress, and inhibiting pro-angiogenic mediators.	Triple Negative Breast Cancer Residual Disease	Phase I Phase II
	TM		Breast Cancer	Phase II
	TM; Exemestane		Breast Cancer	Phase II
	TM		Non-Small Cell Lung Cancer	Phase I
	TM		Prostate Cancer	Phase II
	TM		Prostate Cancer	Phase II
	TM with Radiation or Surgery		Esophageal Carcinoma	Phase II
	TM; bortezomib		Multiple Myeloma	Phase I Phase II
	TM; Temozolomide		Melanoma	Phase II
	TM		Hepatocellular carcinoma	Phase II
	Irinotecan;5-Fluorouracil; Leucovorin; TM		Colorectal Carcinoma	Phase II
Trientine	Trientine dihydrochloride; pegylated liposomal doxorubicin; carboplatin	Exerts anti-copper effects similar to TM in a copper depletion pathway against tumors	Ovarian Neoplasms Malignant (Excl Germ Cell) Peritoneal Carcinoma Fallopian Tube Cancer	Phase I Phase II
	Trientine; Carboplatin; Trientine MTD; Carboplatin MTD		Advanced Cancers	Phase I

Abbreviations: DSF, disulfiram; Abraxane, nab-paclitaxel; AJCC v8, American Joint Committee on Cancer, 8th Edition Cancer Staging Manual; ALDH, aldehyde dehydrogenase; APC, antigen-presenting cell; cGAS-STING, cyclic GMP-AMP synthase-Stimulator of interferon genes; CSCs, ancer stem cells; Doxil, liposomal doxorubicin; EMT, epithelial-mesenchymal transition; ES, elesclomol; Gemzar, gemcitabine; Gemzar, gemcitabine; MTD, Maximum Tolerated Dose; mTOR, Mammalian Target of Rapamycin; p70S6K, p70 S6 Kinase; P-gp, P-glycoprotein; PI3K, Phosphatidylinositol 3-Kinase; RARβ, retinoic acid receptor beta; ROS, reactive oxygen species; TM, tetrathiomolybdate.

DSF [107]. Resistance in melanoma can be broken by DSF through the redox pathway, and in addition, DSF treatment of metastatic melanoma has passed the first phase of clinical trials, bringing new therapeutic hope [211]. Recently, Zhao et al. [212] explored the possibility of "buffet therapy" by utilizing Cu^{2+}-doped hollow zeolite imidazoline framework nanoparticles (HZIFCu) to solve the problem of endogenous Cu^{2+} insufficiency, which provides an idea of DSF in combination with chemotherapy, chemodynamic therapy (CDT), and other therapeutic pathways.

ES was initially included in studies as a mitochondria-targeted anticancer drug and yielded therapeutic value with targeted value for Menkes disease. With the unraveling of the cuproptosis pathway [111], the anticancer mechanism of ES, a copper ion carrier, was expanded again [213]. in vitro treatment of cancer cells with ES induces the rapid production of ROS [108], a cascade reaction that occurs intracellularly causing DNA damage and inducing apoptosis in cancer cells. Similarly, the cytotoxic effects of ES can induce cancer cell death [109], which at the molecular level triggers downstream reactions such as the TCA cycle or FDX1 by translocating cuproptosis into the intracellular compartment. In cancer therapy, ES exhibits high selectivity for cancer categories, those with high mitochondrial metabolism are more likely to show the therapeutic value [213, 214]. Buccarelli et al. [215] used a glioblastoma drug-specific screen to identify ES as the most effective drug, and then combined it with the alkylating agent temozolomide (TMZ) in mice undergoing xenotransplantation for glioblastoma of the brain, which was found to significantly enhance cytotoxicity and demonstrated promising efficacy. Given the strong targeting of mitochondria by ES, it is an excellent performer in breaking through the defenses of chemotherapy-resistant cancer cells that exhibit high mitochondrial metabolism [110, 216]. Therefore, the classification of patients is important for the application of ES precision tumor therapy.

Recently, the polyamine copper chelator is also being developed for cancer treatment when beyond the treatment of diseases like Wilson's disease. After D-penicillamine (DPEN) was shown to sensitize the radiotherapy process via the oxidative stress pathway [217], its anticancer effects were further demonstrated in animal experiments [218], with the use of photochemotherapy CDT in mice. In addition, it has been found that DPEN induces copper aggregation through the upregulation of hCTR1 expression and the downregulation of ATP7A expression, and this anticancer effect can be enhanced when used in conjunction with platinum drugs [219]. Similarly, the concomitant use of trientine with chemotherapeutic agents such as carboplatin demonstrated good tolerability as well as a promising therapeutic outlook, while indicating that serum copper, serum iron, and CP levels may become meaningful aggregation markers [220, 221]. Clioquinol may exhibit anticancer activity through proteasome inhibition [222, 223]. But unfortunately, all of these drugs lack stronger clinical evidence and merely show promise for valuable exploration in therapeutic prospects.

In addition to a wide variety of copper ion carriers, copper-containing compounds and copper nanoparticles can show research value in cancer therapy in a similar pathway. To confirm whether cuproptosis can be induced in CSCs, CuET@PHF nanomaterials were designed to be used in conjunction with photothermal therapy for the treatment of TNBC. Xiao et al. [224] found that they were not only effective in enhancing cuproptosis, but also exhibited elevated immune responses as well as inhibition of tumor recurrence and metastasis. Veeranarayanan's breakthrough discovery of Cu- and Bi- based bimetal chalcogenide (Cu3BiS3) due to its good histocompatibility for ultra-low-dose near-infrared laser-mediated PDT has been well validated, which brings hope for alleviating pain and improving prognostic quality in patient treatment [225]. Guo et al. [226] designed a polymer, PHPM, which helps to induce cuproptosis by altering intracellular copper homeostasis through ROS-triggered copper release after encapsulation of NP@ESCu. Nanoscale metal-organic framework (MOF) Cu-BTC was investigated as a carrier for the in vivo metabolite of DSF, diethyl dithiocarbamate (DDTC), a nanomedicine with potent antitumor effects, Cu-BTC@DDTC, was fabricated and was found to possess a surprising stability and drug loading capacity [226]. Wang et al. [227] designed nanoparticles TPGS/CS-CA for the transport of ES-Cu. This complex enables the bypassing of P-glycoprotein (P-gp), a key target of multidrug resistance (MDR) in cancer cells, demonstrating a new way of thinking about copper nanomedicines [227].

6.2.2 | Copper inhibitors

Copper inhibitors have the same anticancer activity thanks to the bidirectional action of copper, although they exhibit the exact opposite physiological effects of copper supplements. As we mentioned earlier, tumor tissues often exhibit a high copper status, which may imply that copper acts as a trophic factor for tumor growth, proliferation, and migration. Therefore, chelating copper in vivo prevents it from participating in copper metabolism processes, and tumor growth and metastasis-related signaling pathways are inhibited or blocked due to the lack of activation signals. The successful exploration of copper chelator as an anticancer drug in the preliminary stage also proves the rationality of the above reasoning.

TM was used in the early years for the treatment of Wilson's disease, pulmonary fibrosis, and other diseases [228], and with the discovery of toxic side-effects of inhibiting copper ions established on the basis of TM, the knockdown of the copper chaperones Atox1 and CCS has further revealed specific toxicity to tumor cells, including stimulation of oxidative stress as well as modulation of ATP levels without affecting normal cells [229]. The mechanisms and clinical applications of TM have also been further explored, and TM was found to down-regulate the transcription of HIF-1α, a gene involved in tumor angiogenesis, in studies of ovarian and endometrial cancer cell lines [230]. TM was also found to successfully inhibit head and neck squamous cell carcinoma (HNSCC) in an animal model and cause extensive necrosis, particularly affecting tumor metastasis and invasion [231]. It has been surprisingly found that when the chelator is smaller than 6 nm, the drug exhibits an interesting change of state - aggregation into larger secondary particles, which exerts more efficient anti-tumor activity. Therefore, the Cu chelator Imi-Osi has better tumor angiogenesis inhibition and biocompatibility than other copper chelators [232, 233]. In addition, CTR1, a copper transporter protein, has been shown to have a strong correlation with programmed death-ligand 1 (PD-L1), a programmed death receptor, which Zhang et al. [234] utilized to search for the upstream MAPK pathway, opening up the idea of treating breast cancer with cuproptosis chelators [234, 235].

6.2.3 | Combination applications in copper's cancer therapy

In addition to being used alone as an anticancer drug, copper and copper-derived drugs can facilitate the rest of chemotherapy and other means by altering the copper environment of cancer cells to create a tumor microenvironment that is not conducive to their growth and proliferation. This role of combination therapy is called copper adjuvant.

As early as 1983, it was recognized that copper enhances cytotoxicity, and that ascorbic acid produces more reactive oxygen species when copper is present. Based on this principle, mice injected with high doses of copper ascorbate and glycylhistidine were found to show meaningful prolongation of survival in mice inoculated with Ehrlich ascites tumor cells [236, 237]. Paclitaxel is a well-known chemotherapeutic agent that has demonstrated some therapeutic benefits in melanoma treatment. When exploring combinations to improve patient survival, attention has turned to ES, which doubled progression-free survival in combination with paclitaxel and has demonstrated acceptable safety and efficacy in a phase III clinical trial [237, 238]. Recently, Zhao et al. [212] explored the possibility of "buffet therapy" by utilizing HZIFCu to solve the problem of endogenous Cu^{2+} insufficiency, which provides an idea of DSF in combination with chemotherapy, CDT, and other therapeutic pathways. As mentioned previously, copper can target the inflammatory environment of tumors, so it has been enhanced by combining it with anti-inflammatory agents to enhance the induction of DNA damage and activation of the p38 pathway and JNK pathway in breast cancer CSCs [239]. In addition, great promise exists for the application of copper in combination with photochemotherapy. Copper enhances the downstream effects such as reactive oxygen species generated by photostimulation in the human body, emphasizing the tumor inhibitory effect [240].

6.2.4 | Copper therapy is not 100% safe: be vigilant of the toxicity

As a nutrient factor in the body, copper itself is extensively involved in the metabolic processes of the body's organs. Therefore, copper imbalance implies toxicity on a multi-organ, systemic level. The most intuitive negative example of copper overload caused by copper is Wilson's disease. inactivation of ATP 7B leads to copper retention in the liver, causing inflammation and even cirrhosis of the hepatobiliary system [202]. In the nervous system, copper has been reported to accumulate at prominent levels and to appear in higher concentrations in neurological disorders. Higher copper concentrations have been detected in the serum and age spots of patients with Alzheimer's disease (AD) [241]. Similarly, copper accumulation has been seen in Huntington's disease (HD) patients, further inducing huntingtin (HTT) protein aggregation while inhibiting mitochondrial dehydrogenation aggravating the disease [242, 243]. Excess copper also exacerbates the risk of cardiovascular system disease. Serum copper has been shown to correlate with atherosclerosis, and direct measurements of atherosclerotic plaques have revealed locally elevated levels of copper [244, 245]. Völker et al. [246] successfully mimicked atherosclerosis in rat carotid arteries using copper and observed smooth muscle cells, atherosclerosis in the carotid arteries of rats. macrophages, and leucocytes invaded the intima and caused thickening.

In studies of oncological regimens incorporating Cu, a number of trials have reported toxic reactions during the process. Chemotherapy regimens of DSF in combination with copper showed reduced median overall survival (5.5 months) and more adverse events (34% grade 3 and higher adverse events) in patients with recurrent glioblastoma [247]. Another trial comparing the difference in survival between DSF + Cu combination radiotherapy or TMZ con-

ditions showed that no meaningful efficacy was observed in most patients, except for patients with *BRAF*-mutant GBM [248]. A phase II clinical trial of a randomized, double-blind trial of ES in combination with paclitaxel for the treatment of stage IV melanoma demonstrated an increase in median overall survival in the addition of ES group, which was well tolerated [249]. Phase III clinical trials, in which patient data were not screened demonstrated no significant improvement in progression-free survival, with lactate dehydrogenase (LDH) considered a predictive factor, with meaningful improvement demonstrated only when baseline LDH was normal [238, 249]. ES participation in paclitaxel treatment of solid tumors was found to be well-tolerated in combination, with partial remission achieved in the phase I clinical trial, but two patients who had received in-depth treatment showed further toxicity, which the authors analyzed as possibly due to a dose-dependent interaction between ES and paclitaxel [250]. In another trial of ES + Cu in combination with paclitaxel, some patients experienced tertiary toxic reactions, including neutropenia, neurological, thromboembolism, anaemia and metabolic abnormalities [251]. In view of all these, it is necessary to pay close attention to the above disease-related indicators when designing copper therapies, and to be alert to toxic reactions accompanying oncological treatments.

6.3 | Advances in copper-based testing: bioimaging with radioisotopes

In addition to therapeutics, copper has also demonstrated its unique value for some cancers in laboratory science. Positron emission tomography (PET) technology suggests the possibility of cancerous changes in the body by detecting metabolic levels in the body, and the incorporation of the use of novel probes is of great significance for the diagnosis and staging of different cancers. The use of 64 Cu^{2+} as a PET probe has been diagnostically effective in a wide range of cancers including head and neck tumors, prostate, breast, ovarian, liver, colorectal, lung, and others [240]. Grassi et al. [252] conducted a prospective study in 11 patients with head and neck cancer using the probe Cu-diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM), which was found to have high sensitivity and low specificity. The first growth inhibitory receptor imaging (SRI) in humans using 64Cu-DOTATATE showed good image quality and spatial resolution in patients with neuroendocrine tumors and identified 43% additional lesions compared to conventional SRI at a lower radiation dose [253]. Bioimaging based on copper radioisotopes more than 64Cu has been included in research applications, and related studies such as 67Cu have been progressively conducted recently. The design experiment used [64Cu] Cu-SARTATE for pre-treatment PET imaging and irradiated [67Cu] Cu-SARTATE therapeutic volume to this batch of patients with multifocal meningiomas, and guided the treatment by analyzing the results of the PET/CT imaging and achieved a better therapeutic effect [254].

The use of the radioisotope Cu as a molecular probe for PET shows low cost, high safety, and high sensitivity, and is promising, but the diagnostic and therapeutic effective amount of radiation needs to be further explored.

7 | CONCLUSIONS AND PROSPECTS

Is copper our friend or foe? As of now, there's no way to get a completely biased answer. Both mechanistically and therapeutically copper shows a surprising bi-directionality. As an essential trace element, copper intake is important for the maintenance of the nervous system, hematopoiesis, and immunity. The ecology of copper metabolism in the body builds a complex regulatory network, and this protein network shows a surprising degree of overlap with the pathways related to the growth, proliferation, and metastasis of cancer cells, indicating that copper metabolism serves as a meaningful target for anticancer drugs.

At the same time, however, it is noteworthy that the intracellular circulatory pathways of copper metabolism have not been fully explored, and the definition of the mechanism of cuproptosis is yet to be further refined. In addition, the high similarity and correlation between cuproptosis and ferroptosis implies that anti-cancer therapeutic ideas based on ferroptosis can be attempted to be reproduced on cuproptosis. The complex landscape built out of cuproptosis and other cell death pathways also suggests additional possibilities for anticancer therapy. It has been demonstrated that copper is involved in the construction of the immune environment and the induction of inflammatory responses, but experimental evidence based on this principle is lacking. Copper is involved in various pathways of tumor proliferation and metastasis, but the specific intervention mechanism needs to be further elaborated at the molecular level, which will also have a complementary and guiding significance to the pharmacological effects of drugs. Although sCP is the most widely used means of detecting copper status, it still has the defects of poor sensitivity and easy to be interfered by factors such as inflammation, liver disease, pregnancy, etc. The development of new means of measurement is still meaningful for cancer diagnosis, typing and staging. In the exploration of anticancer drugs with different drug properties, nanotechnology, radionuclides, and photochemical kinetic therapy show good compatibility for copper-related drugs, which implies that copper-based cancer therapy can

break through the traditional copper ion carriers and copper chelating agents and obtain better therapeutic efficacy through the new design of compounds.

AUTHOR CONTRIBUTIONS

Zaoqu Liu and Yuqing Ren provided direction and guidance throughout the preparation of this manuscript. Jinling Song, Yuqing Ren, and Dan Shan wrote and edited the manuscript. Jinhai Deng and Dan Shan reviewed and made significant revisions to the manuscript. Xinwei Han, Zaoqu Liu, and Jinhai Deng revised and edited the manuscript. Yuyuan Zhang, Yuhao Ba, Jinhai Deng, Peng Luo, Quan Cheng, Hui Xu, Siyuan Weng, Anning Zuo, and Shutong Liu collected and prepared the related papers. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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