REVIEW

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Targeting cuproptosis for cancer therapy: mechanistic insights and clinical perspectives

Chenliang Zhang[1*†], Tingting Huang[2†] and Liping Li[3]

Abstract

Cuproptosis is a newly identified form of cell death induced by excessive copper (Cu) accumulation within cells. Mechanistically, cuproptosis results from Cu-induced aggregation of dihydrolipoamide S-acetyltransferase, correlated with the mitochondrial tricarboxylic acid cycle and the loss of iron–sulfur cluster proteins, ultimately resulting in proteotoxic stress and triggering cell death. Recently, cuproptosis has garnered significant interest in tumor research due to its potential as a crucial therapeutic strategy against cancer. In this review, we summarized the cellular and molecular mechanisms of cuproptosis and its relationship with other types of cell death. Additionally, we reviewed the current drugs or strategies available to induce cuproptosis in tumor cells, including Cu ionophores, small compounds, and nanomedicine. Furthermore, we targeted cell metabolism and specific regulatory genes in cancer therapy to enhance tumor sensitivity to cuproptosis. Finally, we discussed the feasibility of targeting cuproptosis to overcome tumor chemotherapy and immunotherapy resistance and suggested future research directions. This study suggested that targeting cuproptosis could open new avenues for developing tumor therapy.

Keywords Copper, Cuproptosis, Cancer, Tumor therapy, Drug resistance, Tumor immunotherapy

Introduction

Copper (Cu) is an essential trace metal element for normal physiological functions primarily obtained from dietary supplements. In biological systems, Cu exists predominantly in two oxidative states: divalent copper ions (Cu^{2+}) and monovalent copper ions (Cu^+). Cu^+ is the principal oxidative form and plays a significant role

in physiological and pathological regulation within cells [1–3]. Disruptions in Cu homeostasis can induce disease onset; for instance, Cu overload may lead to Wilson's disease [4], while Cu deficiency can cause Menkes disease [5]. Furthermore, previous studies have demonstrated that Cu could promote tumor cell proliferation, angiogenesis, and metastasis and reduces the efficacy of tumor treatments [6].

However, excess Cu in the cells can also cause cellular damage. Researchers observed that using Cu ionophores to elevate Cu levels within tumor cells could cause cell death [7, 8]. Although subsequent studies have extensively investigated the molecular mechanisms underlying Cu-induced cell death, such as the association of this type of cell death with reactive oxygen species (ROS), apoptosis, and ferroptosis-related signaling pathways, the key mechanisms remain unclear. Tsvetkov et al. termed the Cu-induced cell death cuproptosis in 2022 based on their findings that this form of cell death depends on the aggregation of lipoylated dihydrolipoamide S-acetyltransferase (DLAT) and the reduction of iron–sulfur cluster (Fe–S)

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proteins, triggered by Cu accumulation in mitochondria, leading to proteotoxic stress, and cell death [9].

With the concept and mechanism of cuproptosis established, researchers have increasingly focused on cuproptosis in cancer therapy and demonstrated that Cu-based treatments play a pivotal role in inhibiting tumor growth. In this review, we summarized the core molecular mechanisms of cuproptosis and discussed the relationship between it and other forms of cell death. Moreover, we systematically summarized the current understanding of targeting cuproptosis for tumor therapy, including using Cu ionophores, small compounds, and nanomedicine to induce cuproptosis and targeting cell metabolism or certain genes to sensitize cuproptosis. Additionally, we discussed the potential of targeting cuproptosis to overcome tumor drug resistance in chemotherapy, targeted therapy, and immunotherapy. We also discussed the opportunities and challenges in targeting cuproptosis-associated cancer therapy.

Cu metabolism

Cu homeostasis is essential for the normal physiological functioning of human life. In humans, Cu uptake, distribution, transport, and elimination are meticulously regulated (Fig. 1A), which is crucial to prevent deficiency or excessive Cu accumulation in various tissues and cells, thereby averting disease onset [10]. Humans predominantly acquire Cu through their diet, with an adult daily requirement ranging from 0.8 to 2.4 mg [3]. Following digestion in the stomach and duodenum, Cu is primarily absorbed in the small intestine, where Cu^{2+} is reduced to Cu^+ by metalloreductases, such as six-transmembrane epithelial antigen of the prostate (STEAP) [11] and duodenal cytochrome b (DCYTB) [12], before being transported into enterocytes by Cu transport protein 1 (CTR1), also known as the solute carrier family 31 member 1 (SLC31A1) [13], located at the apical membrane of enterocytes. Subsequently, Cu is exported to the interstitial fluid or bloodstream by the protein ATPase copper transporting alpha (ATP7A) [14]. Cu in the bloodstream usually binds to plasma proteins, such as ceruloplasmin and human serum albumin, and is transported to the liver, the primary Cu storage organ, via the portal system [15]. Metallothionein1/2 (MT1/2), a thiol-rich protein, binds to and stores Cu in hepatocytes [16]. Hepatocytes can excrete excess Cu into the bile via ATP7B. Beyond the liver, Cu in the bloodstream can also be absorbed by other tissues and organs, including the heart and the brain [15].

After being transported into cells by transmembrane proteins, such as CTR1 and divalent metal transporter 1 (DMT1), Cu can be stored by binding to intracellular Cu chaperones, such as MT1/2 and glutathione (GSH),

thereby preventing cellular damage [1, 17]. Concurrently, Cu can be delivered to other cellular structures or proteins via Cu chaperones to maintain normal cellular function (Fig. 1B). For example, the Cu chaperone for superoxide dismutase (CCS) binds to Cu and delivers it to superoxide dismutase 1 (SOD1), catalyzing the conversion of superoxide radicals to H_2O_2, thus maintaining cellular ROS homeostasis [18]. Abnormal SOD1 expression is considered to be linked to tumor development [19]. Additionally, the Cu chaperone antioxidant 1 (ATOX1), which binds Cu^+ via two cysteine residues, can transport cytosolic Cu^+ to the ATP7A/ATP7B, located in the Golgi network [20, 21]. Excess Cu^+ enters the trans-Golgi network (TGN) and can be expelled from the cell via the vesicular system [20, 21]. The absence of ATOX1 leads to perinatal lethality induced by Cu dyshomeostasis [22]. Moreover, increased intracellular Cu levels can promote the distribution of ATP7A and ATP7B within the TGN, thereby facilitating Cu efflux [23]. These studies highlight significant regulatory roles of ATOX1 and ATP7A/B in intracellular Cu homeostasis.

The mitochondria are the primary targets of intracellular Cu (Fig. 1B). Cu contributes to ATP production within the mitochondria by ensuring the catalytic function of cytochrome oxidase (COX) during oxidative phosphorylation (OXPHOS). Cytosolic Cu^+ can be transported to the mitochondrial intermembrane space by a Cu ligand (CuL), a non-proteinaceous low-molecular-weight complex, where it can enter the mitochondrial matrix via solute carrier family 25 member 3 (SLC25A3) located in the inner mitochondrial membrane (IMM) [24, 25]. Cu in the matrix is transported back across the IMM to intermembrane space (IMS) by an unknown transporter, where it is delivered to COX and SOD1 [26]. Additionally, COX17 can transfer cytosolic Cu^+ to the mitochondrial intermembrane space and deliver Cu^+ to other Cu chaperone molecules, such as COX11 and synthesis of cytochrome c oxidase 1/2 (SCO1/2), which then deliver Cu^+ to COX1 and COX2, respectively [27, 28].

Cu and cancer

The role of Cu in tumorigenesis and tumor therapy has been of great concern. Compared to the healthy population, elevated Cu levels have been observed in the tumors or serum of patients with various types of cancers, including breast [29, 30], prostate [31], lung [32], cervical [33], and bladder [34], thyroid [35], and oral cancers [36, 37]. Cu can promote the onset and development of tumors, termed cuproplasia, by activating oncogenic signaling pathways [6]. For instance, Cu can directly bind mitogen-activated extracellular signal-regulated kinase (MEK) and activate its downstream mitogen-activated protein kinase (MAPK) pathway to promote tumor cell growth [38, 39].
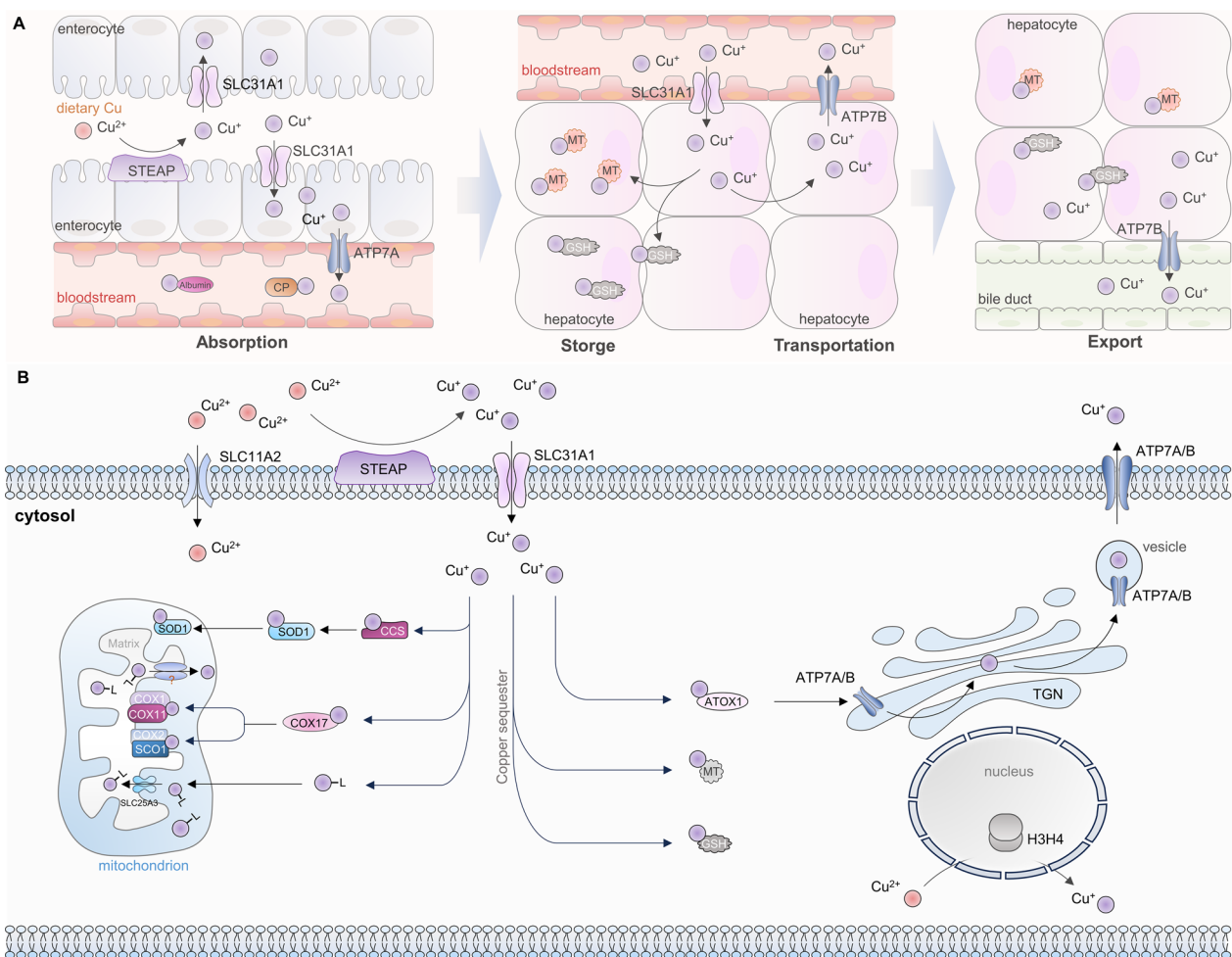
Fig. 1 Cu metabolism. A. Cu homeostasis in physiological systems. Dietary Cu^{2+} is reduced to Cu^+ by the STEAP family members and subsequently transported into enterocytes via SLC31A1. Within enterocytes, Cu^+ is released into the bloodstream by ATP7A, where it typically binds to soluble chaperones such as albumin and ceruloplasmin. Hepatocytes uptake Cu^+ from the bloodstream through SLC31A1 on their cell membrane. In hepatocytes, Cu^+ can be delivered to MT or GSH for storage, or to ATP7B for re-entry into the bloodstream for uptake by other tissues and organs. Additionally, ATP7B in hepatocytes can excrete excess Cu^+ into the bile duct. **B.** Cu metabolism at the cellular levels. Extracellular Cu^+ is taken up by SLC31A1 on the cell membrane, while Cu^{2+} can be transported into the cell by SLC11A2. Intracellular Cu^+ can be sequestered by MT1/2 and GSH or bound to other Cu chaperones such as CCS, COX17, and ATOX1 for further trafficking to proteins. CCS delivers Cu^+ to cytosolic SOD1, COX17 transports Cu^+ to mitochondrial COX11 or SCO1, and ATOX1 delivers Cu^+ to ATP7A/B located on the Golgi membrane in the TGN, ultimately secreting it outside the cell. Cu^+ can also form a non-proteinaceous low-molecular-weight complex, CuL, which transports Cu^+ into mitochondrion. Cu^+ can be transported into the mitochondrial matrix via solute carrier family 25 member 3 (SLC25A3) located in inner mitochondrial membrane. Cu in the matrix is transported back across the IMM to intermembrane space (IMS) by an unknown transporter. Histones H3/H4 in the nucleus can reduce Cu^{2+} to Cu^+. STEAP, six-transmembrane epithelial antigen of the prostate; SLC31A1, the solute carrier family 31 member 1; ATP7A, ATPase copper transporting alpha; MT1/2, Metallothionein1/2; GSH, glutathione; CCS, Cu chaperone for superoxide dismutase; COX, cytochrome oxidase; SOD1, superoxide dismutase 1; ATOX1, antioxidant 1; TGN, trans-Golgi network; SLC25A3, solute carrier family 25 member 3

In melanoma driven by $BRAF^{V600E}$, chelating Cu can inhibit MAPK signal and reduce tumor cell resistance to $BRAF^{V600E}$ and MEK1/2 inhibitors [40]. Moreover, Cu is considered to contribute to tumor metastasis. Cu can act on metalloenzymes in the extracellular matrix, such as lysyl oxidase (LOX) [41] and secreted protein acidic and rich in cysteine (SPARC) [42], altering cell–matrix and cell–cell interactions, thereby promoting tumor cell migration and metastasis. In breast cancer cells, Cu can bind to the mediator of ErbB2-driven cell motility 1 (MEMO1) and activate its oxidase activity promoting O^{2-} production and enhancing tumor cell migration and invasion [43, 44]. Additionally, Cu can promote angiogenesis, essential for tumor growth and progression,

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by activating pro-angiogenic factors, such as fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF), tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, and IL-8 [45–48]. Taken together, Cu is a critical trace metal element in tumor development. Elucidating the molecular mechanisms by which Cu drives tumorigenesis will aid the discovery of new therapeutic targets for cancer.

Cuproptosis

Although Cu-induced cell death has been investigated for decades, the concept of 'cuproptosis' was not proposed until 2022 by Tsvetkov and colleagues [9]. A significant feature of Cu ionophores and Cu-treated cells is a sharp increase in ROS levels, which has long been considered the main cause of cell death. ROS scavengers, such as *N*-acetylcysteine (NAC), can mitigate the extent of Cu-induced cell death in some cells [49–53]. However, eliminating ROS does not always inhibit cell death induced by Cu [9, 54], suggesting that ROS are not primary mediators of Cu-induced cell death. Tsvetkov et al. also observed that antioxidants, such as NAC, JP4-039, ebselen, and α-tocopherol, were unable to rescue cells from elesclomol (a Cu ionophore)–Cu-induced cellular damage, whereas agents that chelate Cu, such as GSH and ammonium tetrathiomolybdate (TTM), could prevent the lethality of elesclomol–Cu to cells [9]. This type of cellular damage could not be rescued by inhibitors of other types of cell death, such as apoptosis, ferroptosis, and necrosis. Tsvetkov et al. found that elesclomol could transport Cu^{2+} across membranes into the mitochondria, where Cu^{2+} was reduced to Cu^+ by ferredoxin 1 (FDX1) [9], a mitochondrial matrix reductase. However, excess mitochondrial Cu^+ can directly bind to lipoylated DLAT, an essential component of mitochondrial TCA cycle, causing DLAT aggregation and cytotoxicity [9]. Additionally, excess mitochondrial Cu^+ reduces the stability of the Fe–S cluster proteins [9], which play an important role in OXPHOS-related electron transport. These induce proteotoxic stress, ultimately leading to cell death (Fig. 2). Pharmacological inhibition of the electron transport chain (ETC) and pyruvate uptake could reverse elesclomol–Cu-induced cell death [9], indicating that cuproptosis depends on mitochondrial respiration. Additionally, whole-genome CRISPR-Cas9 screening, combined with single-gene knockout validation experiments, showed that proteins in the lipoic acid (LA) pathway, such as FDX1, LA synthase (LIAS), lipoyl transferase 1 (LIPT1), and dihydrolipoamide dehydrogenase (DLD), and genes in the pyruvate dehydrogenase complex, such as DLAT, pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1), pyruvate dehydrogenase E1 subunit beta (PDHB), metal-regulatory transcription factor-1

(MTF1), glutaminase (GLS), and cyclin-dependent kinase inhibitor 2A (CDKN2A), are important regulators of cuproptosis (Fig. 2) [9]. These results also ascertain the significance of FDX1 and its regulation of mitochondrial protein lipoylation in cuproptosis.

Cuproptosis and regulated cell death

Although Cu ionophores combined with Cu can induce cuproptosis, previous studies have also found that excessive Cu in cells can cause other ways of regulated cell death (RCD) (Fig. 3), suggesting a molecular mechanism crosstalk between cuproptosis and other forms of cell death. Understanding the role of Cu in other cell death modes can help develop more reasonable tumor treatment strategies targeting cuproptosis.

Cuproptosis and ferroptosis

Ferroptosis is a form of programmed cell death induced by disrupting iron homeostasis and accumulating ROS in lipids. Although Tsvetkov and colleagues found that the ferroptosis inhibitor ferrostatin-1 did not rescue cells from growth inhibition induced by elesclomol–Cu, Gao et al. observed that ferrostatin-1 and liproxstatin-1, another ferroptosis inhibitor, could inhibit elesclomol–Cu-induced cell damage in colorectal cancer (CRC) cells [55]. Gao et al. demonstrated that elesclomol alone treatment could reduce the ATP7A expression levels in CRC cells, potentially mediating the degradation of solute carrier family 7 membrane 11 (SLC7A11), a cysteine-glutamate transporter, resulting in lipid peroxidation and ferroptosis, suggesting that it is unclear whether elesclomol–Cu-induced ferroptosis depends on Cu [55]. Furthermore, previous studies have reported that reducing the Fe–S cluster proteins, which are the main features of cuproptosis, can induce ferroptosis [56]. Cu can exacerbate erastin-induced ferroptotic cell death in pancreatic ductal adenocarcinoma (PDAC) cells through increasing ubiquitination and aggregation of glutathione peroxidase 4, a protein blocking ferroptosis by eliminating phospholipid hydroperoxides, promoting its macroautophagic degradation [57].

These studies indicate a close interplay between cuproptosis and ferroptosis, with a key intersection being mitochondrial metabolism. As essential energy sources for cells, mitochondria play critical roles in regulating ferroptosis [58]. Cellular energy metabolic pathways, such as the TCA cycle and glycolysis, are involved in this regulation. For instance, blocking the TCA cycle or loss of glutamine can attenuate cystine-deprivation or erastin-induced ferroptosis [59], suggesting that the TCA cycle and glutaminolysis are required for ferroptosis. Similarly, in the process of cuproptosis, the TCA cycle in mitochondria is crucial [9]. The aggregation of
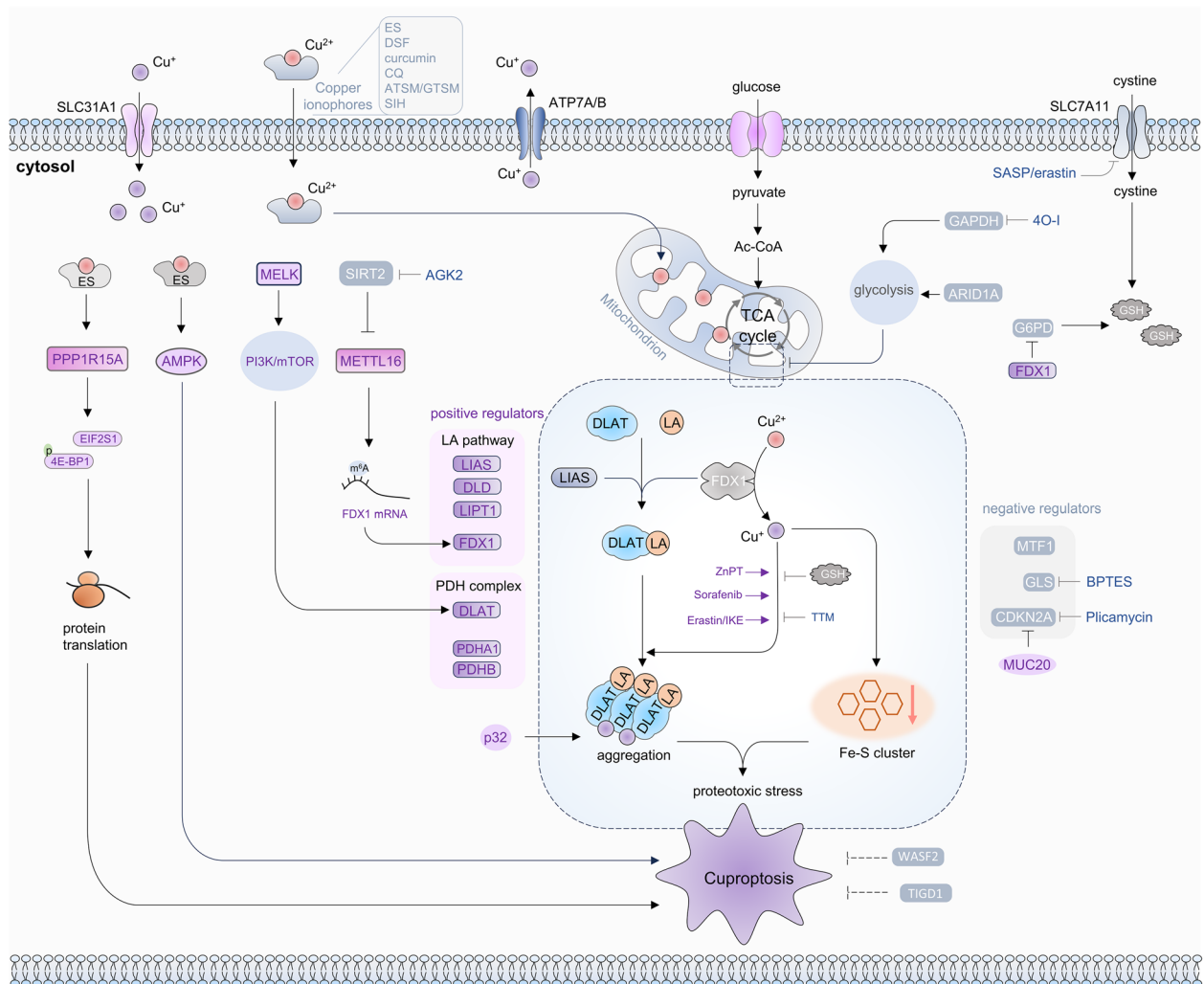
Fig. 2 Mechanisms of cuproptosis. The transportation of Cu^{2+} by Cu ionophores and the uptake of Cu^+ by SLC31A1 lead to the excessive accumulation of Cu within cells. Cu ionophores, such as elesclomol, can transport Cu^{2+} into mitochondria, where Cu^{2+} is reduced to Cu^+ by FDX1. FDX1 is a crucial regulatory protein for the lipoylation of mitochondrial TCA cycle enzymes, particularly DLAT. Accumulated Cu^+ in mitochondria induces DLAT aggregation by directly binding to lipoylated DLAT and destabilizes Fe–S cluster proteins, ultimately triggering mitochondrial proteotoxic stress and resulting in cuproptosis. Key positive regulators of cuproptosis include LIAS, DLD, LIPT1, and FDX1 from the LA pathway, as well as DLAT, PDHA1, and PDHB from the PDH complex. Important inhibitors of cuproptosis include MTF1, GLS, and CDKN2A. FDX1 promotes G6PD degradation by binding to it, resulting in GSH reduction and intensified cuproptosis. METTL16 enhances cuproptosis by promoting FDX1 accumulation via m6A modification on *FDX1* mRNA, a process inhibited by SIRT2 through delactylating METTL16 at K229. MELK increases DLAT expression through the PI3K/mTOR signaling pathway, enhancing mitochondrial function and cuproptosis. AMPK activated by elesclomol–Cu facilitates cuproptosis. Elesclomol–Cu upregulates PPP1R15A to promote proteotoxic stress by enhancing EIF2S1 and 4E-BP1-associated translation initiation, thereby enhancing cuproptosis. p32 enhances elesclomol–Cu-induced cuproptosis by promoting lipo-DLAT oligomerization. MUC20 induces cuproptosis by inhibiting CDKN2A expression. GAPDH and ARID1A inhibit cuproptosis by promoting cellular glycolysis, while SLC7A11 inhibits cuproptosis by upregulating intracellular GSH. SLC31A1, the solute carrier family 31 member 1; FDX1, ferredoxin 1; ES, elesclomol; Disulfiram, disulfiram; DLAT, dihydrolipoamide S-acetyltransferase; TCA, tricarboxylic acid; LIAS, LA synthase; LIPT1, lipoyl transferase 1; DLD, dihydrolipoamide dehydrogenase; PDHA1, pyruvate dehydrogenase E1 subunit alpha 1; PDHB, pyruvate dehydrogenase E1 subunit beta; MTF1, metal-regulatory transcription factor-1; GLS, glutaminase; CDKN2A, cyclin-dependent kinase inhibitor 2A;G6PD, glucose-6-phosphate dehydrogenease; GSH, glutathione; SIRT2, Sirtuin 2; SLC7A11, solute carrier family 7 membrane 11; MUC20, Mucin 20; MELK, maternal embryonic leucine zipper kinase; ARID1A, AT-rich interactive domain 1A; AMPK, adenosine 5′-monophosphate (AMP)-activated protein kinase; TIGD1, trigger transposable element-derived 1; WASF2, Wiskott-Aldrich syndrome protein family member 2

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lipoylated DLAT in the TCA cycle is a major inducer of elesclomol–Cu-mediated cell death [9]. Furthermore, cysteine-deprivation-induced ferroptosis is affected by α-ketoglutarate and other intermediates of the TCA cycle, such as succinate and fumarate [58]. Inhibition of ETC complexes can attenuate cystine-deprivation and erastin-induced lipid peroxidation and cell death [59]. Interestingly, inhibition of ETC complexes can also mitigate elesclomol–Cu-induced cuproptosis [9], suggesting that cuproptosis and ferroptosis share the same mitochondrial energy dependency.

Another important hub for cuproptosis and ferroptosis is GSH, a crucial antioxidant. GSH acts as an inhibitor of both ferroptosis and cuproptosis, indicating that it serves as a significant co-regulator in these processes. Both Cu and iron (Fe) promote the oxidation and subsequent consumption of GSH by binding to it [60]. Additionally, GSH chelates Fe and Cu to reduce metal ion toxicity [60]. Since Fe can consume GSH, over accumulation of Fe in cells may trigger cuproptosis by inhibiting GSH. GSH is also a necessary cofactor for GPX4, which reduces cytotoxic lipid peroxides (L-OOH) to corresponding alcohols (L-OH) while converting reduced GSH to oxidized glutathione (GSSG), thus reducing lipid peroxidation and inhibiting ferroptosis [61]. The accumulation of Cu in cells promotes the consumption of GSH, creating favorable conditions for ferroptosis. SLC7A11 transports glutamate outside and cystine inside cells. Cystine is then converted to cysteine, a component of GSH. SLC7A11 inhibitors, such as sorafenib and erastin, are commonly used to induce ferroptosis by reducing intracellular cysteine

levels and GSH synthesis, which also makes cuproptosis more likely. Indeed, Wang et al. recently observed that sorafenib and erastin can induce cuproptosis in primary liver cancer cells by primarily reducing intracellular GSH synthesis and increasing Cu-dependent lipoylated protein aggregation [62]. Additionally, BSO, an inhibitor of GSH synthesis known to induce ferroptosis, has also been found to induce cuproptosis [9]. These studies indicate that GSH is a critical molecule mediating the crosstalk between ferroptosis and cuproptosis. Targeting GSH could be a potential strategy to simultaneously induce ferroptosis and cuproptosis in tumor cells.

ROS are also critical factors in understanding the crosstalk between ferroptosis and cuproptosis. Both Fe and Cu can produce ROS through the Fenton reaction, while rapid GSH depletion mediated by these metals can further exacerbate cellular ROS accumulation. Excessive ROS promotes lipid peroxidation and ferroptosis [63]. Although in elesclomol–Cu-induced cuproptosis, Cu toxicity primarily results from the aggregation of lipoylated proteins in the mitochondria rather than ROS production [9], excessive Cu-mediated ROS generation and GSH depletion can also contribute to ferroptosis onset. In summary, while cuproptosis and ferroptosis have distinct initiation mechanisms and molecular characteristics, they mutually influence each other, creating favorable conditions for both processes. This interplay adds complexity to their regulatory mechanisms but also offers potential advantages for cancer therapy.

(See figure on next page.)

Fig. 3 Cuproptosis and regulated cell death. A Excess Cu triggers ferroptosis. Accumulation of Cu in mitochondria generates ROS, which promotes lipid peroxidation and induces ferroptosis. Cu enhances the ubiquitination of GPX4, a protein that blocks ferroptosis by eliminating phospholipid hydroperoxides, facilitating its autophagic degradation and exacerbating ferroptotic cell death. Ferroptosis inducers, such as sorafenib and erastin, can induce cuproptosis by upregulating FDX1 protein levels, promoting lipoylated protein aggregation, and downregulating GSH. The reduction of Fe–S cluster proteins mediated by excessive Cu in mitochondrion further promotes ferroptosis. **B** Excess Cu triggers apoptosis. Intracellular accumulation of Cu generates ROS via the Fenton reaction, which induces apoptosis. Mitochondrial Cu accumulation causes mitochondrial stress, leading to the localization of pro-apoptotic proteins (such as BAX and BAK) to the outer mitochondrial membrane, resulting in the release of cytochrome c from mitochondria. Cytosolic cytochrome c induces the formation of the apoptosome, which activates the caspases signaling axis, mediating apoptosis. Cu can inhibit proteasome activity either by directly binding to the proteasome or by causing NPL4/p97 aggregation, inducing ER stress and ultimately leading to apoptosis. Additionally, Cu can activate the MAPK-JNK signaling pathway to trigger cell apoptosis. **C** Cu regulates autophagy. Cu activates ULK1/2 by directly binding to them, promoting phagophore assembly and subsequently autophagosome formation. Cu can also inhibit mTOR by activating AMPK, facilitating phagophore formation. Excess Cu in cells upregulates the expression of autophagy-related genes, such as MAP1LC3 and ATG5. Cu inhibits the cysteine protease activity of ATG4B by directly binding to it, thus preventing the delipidation of MAP1LC3 and consequently blocking cellular autophagy. Cu promotes the fusion of lysosomes and autophagosomes, enhancing cellular autophagic flux. **D.** Excess Cu induces pyroptosis. Cu induces ROS production and ER stress, promoting the formation of the NLRP3 inflammasome, which activates caspase 1. Caspase 1 cleaves GSDMD to generate the N-terminal domain that creates membrane pores, promoting pyroptosis. ROS, reactive oxygen species; GPX4, glutathione Peroxidase 4; FDX1, ferredoxin 1; GSH, glutathione; ER, endoplasmic reticulum; BAX, BCL2 Associated X; BAK, BCL2 antagonist/killer 1; cyto.c, cytochrome c; MAPK, mitogen-activated protein kinase; JNK, Jun N-terminal kinase; ULK1/2, Unc-51-like autophagy activating kinase 1/2; mTOR, mechanistic target of rapamycin kinase; AMPK, adenosine 5 '-monophosphate (AMP)-activated protein kinase; MAP1LC3, microtubule-associated protein 1 light chain 3; ATG5, autophagy related 5; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; GSDMD, gasdermin D
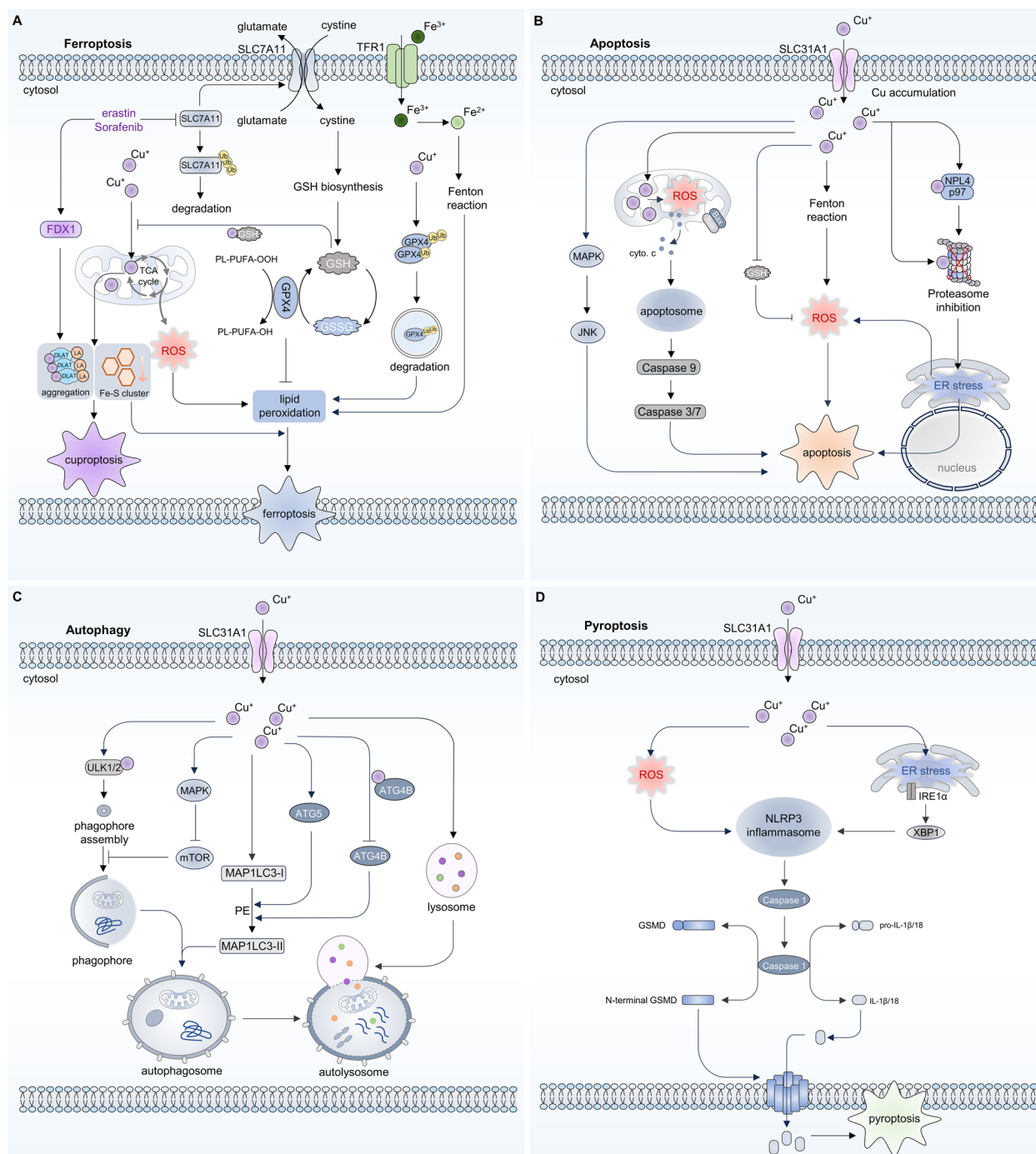
Fig. 3 (See legend on previous page.)

Cuproptosis and apoptosis

Excessive Cu accumulation in cells has also been linked to apoptosis. For instance, treatment with $CuSO_4$ can upregulate the C/EBP homologous protein (CHOP), Jun N-terminal kinase (JNK), and caspase-12 expression levels in mouse liver cells, thereby enhancing cell apoptosis-related signaling pathways, such as endoplasmic reticulum (ER) stress [64]. Liu et al. also found that treatment with $CuSO_4$ can increase the ROS levels and protein carbonyl compounds in cells and decrease GSH levels, thereby activating the mitochondrial pathway of apoptosis signaling, such as cytochrome c release into the cytosol and cleavage of caspase-9 and caspase-3 [65]. In myeloma cells and osteosarcoma, disulfiram, another

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Cu ionophore, combined with Cu, can induce apoptosis by activating ROS and JNK signaling pathways [66, 67]. Furthermore, Cu can induce apoptosis by binding to and inhibiting 20S proteasome subunits and activating the cytochrome c-caspase cascade signaling axis [68, 69].

Before the identification of cuproptosis, the damage to tumor cells treated with Cu ionophores combined with Cu was primarily considered to result from ROS production. Excessive ROS within cells can trigger apoptosis through various pathways, including ER stress, mitochondrial damage, and activation of death receptors. In certain tumor cells, such as lung cancer cells [49, 51], gastric cancer [50], melanoma [52], and osteosarcoma [53], ROS scavengers can mitigate the cell damage induced by Cu ionophores and Cu. However, in breast cancer and glioblastoma cells [9, 54], ROS scavengers do not exhibit this protective effect. Additionally, some studies have demonstrated that Cu indeed induces apoptosis in specific tumor cells [52, 67, 70]. These studies suggest that ROS may serve as a crucial link between cuproptosis and apoptosis. Additionally, different tumor cells exhibit varying tolerance and responses to ROS, which may explain the diverse roles of apoptosis in Cu-induced cell death.

Cuproptosis and autophagy

Cu is also considered to regulate autophagy. Studies have revealed that Cu can upregulate the autophagy-related gene expression in cells, such as *LC3b/LC3a*, *BECN1*, *Atg3*, and *Atg5* [71, 72]. Besides, Cu can directly bind to the Unc-51-like autophagy activating kinase 1/2 (ULK1/2), crucial protein kinases regulating autophagy initiation, activating ULK1/2 and its downstream autophagy pathway [73]. In KRAS^{G12D}-driven lung cancer, deletion of the Cu transporter SLC31A1 diminished the Cu-mediated activation of ULK1/2, resulting in a blockage in the autophagic flux and tumor growth suppression [73]. However, whether Cu-induced autophagy contributes to Cu toxicity remains uncertain. For instance, Tang et al. found that in ATP7B R778L mutant hepatocytes, Cu can activate autophagy, which is beneficial for inhibiting cell necrosis and reducing Cu toxicity [74]. However, in some tumor cells Cu was found to inhibit autophagy. For instance, inhibiting SLC31A1-dependent copper absorption could enhance autophagic flux of pancreatic cancer cells, leading to the suppression of tumor cell death [75]. Besides, it was found that Cu could directly bind to autophagy-related gene 4B, a crucial regulator in the autophagy process responsible for priming and delipidation of LC3, and suppress its cysteine protease activity, consequently blocking cellular autophagy [76]. These results indicate that the regulation of the autophagy process by Cu is bidirectional.

Cuproptosis and pyroptosis

Moreover, Cu can affect pyroptosis in cells. In jejunal epithelial cells, Cu can upregulate the pyroptosis-related gene expression, such as *CASP1*, *GSDMD*, and *IL-1β*, which is believed to be mainly mediated by the ER stress-triggered IRE1α-XBP1 pathway [77]. In hepatocytes, Cu can similarly upregulate pyroptosis-related gene expression, such as *CASP1*, *NLRP3*, *IL-1β*, and *IL-18*, and NAC and a caspase inhibitor can reverse this behavior, suggesting that ROS generated by Cu induction may be the main mediators of pyroptosis [78]. Excessive ROS can induce various types of cell death, such as apoptosis, ferroptosis, pyroptosis. Cu can induce ROS generation in numerous tumor cells, which may be a crucial factor in the cross-talk between different types of Cu-triggered RCD. This indicates that in some tumor cells highly sensitive to ROS, cuproptosis may not be irreplaceable in Cu-induced cell damage. In summary, Cu overload can cause cellular damage from multiple angles. Although this complicates the mechanism of Cu-induced cell damage, it provides more possibilities for Cu-based tumor therapy.

Targeting cuproptosis for cancer therapy

Although Cu promotes tumorigenesis to a certain extent, excessive Cu accumulation in tumor cells disrupts cellular homeostasis and induces cuproptosis. Therefore, targeting cuproptosis may be a potential tumor treatment strategy.

Induction of tumor cell cuproptosis
Cu ionophores

Cu ionophores, defined as compounds or chemicals, can bind to Cu and carry it into cells, increasing the intracellular Cu levels. As previously mentioned, the most extensively studied Cu ionophores are elesclomol and disulfiram (Table 1). Elesclomol, a highly lipophilic Cu-binding molecule, can chelate extracellular Cu^{2+} to form an elesclomol–Cu^{2+} complex, facilitating the transport of Cu into cells [145, 146]. The anti-tumor activity of elesclomol has been recognized for decades and is believed to be Cu-dependent (Table 1). Although early studies reported that cell death induced by elesclomol–Cu was linked to apoptosis and ferroptosis, Tsvetkov et al. observed that elesclomol–Cu induced cellular damage through cuproptosis [9]. Elesclomol–Cu can increase ROS levels in tumor cells [49, 54, 83], exacerbating cellular damage and suggesting that elesclomol–Cu may kill tumor cells via multiple pathways. Additionally, recent studies have revealed FDX1-independent mechanism(s) of elesclomol–associated Cu release, achieving Cu delivery to non-mitochondrial cuproproteins [147]. Whether these non-mitochondrial Cu participates in cell death other than cuproptosis induced by elesclomol–Cu

Table 1 The anti-tumor effect of copper ionophores

Compound	Cancer type	Materials (cell lines)	Combined drugs	Anti-tumor effect and its involved mechanism	Refs.
Elesclomol	Lung cancer	SCLCSR2, SCLCBC, NSCLCSC, NSCLCFC		Elesclomol selectively induces cytotoxicity in cisplatin-resistant lung cancer cells by enhancing ROS generation	[49]
Elesclomol	Cervical cancer	Hela		The antitumor efficacy of elesclomol can be enhanced by down-regulating nucleus accumbens-1	[79]
Elesclomol	Breast cancer	MCF-7, MDA-MB-231, HCC1806	DOX, paclitaxel	Elesclomol alone modestly inhibits the growth of human breast cancer cells. Elesclomol enhances DOX- or paclitaxel-induced apoptosis and suppresses breast cancer cell growth, partially mediated by the JNK1 pathway	[80]
Elesclomol	GBM	GSCs, GdECs	TMZ	Elesclomol induces a significant increase in mitochondrial ROS in both GSCs and GdECs cells, resulting in a non-apoptotic Cu-dependent cell death. Combined treatment with elesclomol and TMZ enhances the antitumor efficacy of TMZ both in vitro and in vivo	[54]
Elesclomol	Leukemia, lymphoma	HSB2, Ramos		Elesclomol induces apoptosis in cancer cells through triggering oxidative stress	[81]
Elesclomol	Ovarian cancer	RMG1, OVCA432	ARID1A siRNA	Knockdown of ARID1A expression increases the sensitivity of ovarian cancer cells to elesclomol treatment	[82]
Elesclomol	Uveal melanoma	GNAQ/11-mutant cells	Binimetinib	Elesclomol selectively suppresses GNAQ/11-mutant uveal melanoma by generating excess ROS, leading to YAP inactivation and SNAI2 downregulation. The combination of elesclomol and binimetinib synergistically inhibits the growth of binimetinib-resistant uveal melanoma cells	[83]
Elesclomol	Breast cancer	BRCA1-mutated breast cancers cells, Basal-like breast cancers cells		BRCA1-mutated and/or basal-like breast cancers cells with defective base-excision repair of oxidative DNA damage are highly sensitive to elesclomol	[84]
Elesclomol	Lung cancer	A549, Calu-1		Elesclomol treatment induces the expression of apoptotic proteins and inhibits the expression of metastatic proteins, as well as cell migration, in lung cancer cells. Additionally, elesclomol treatment decreases p-ERK activity while increasing p-Akt levels	[85]
Elesclomol/Cu	Melanoma, leukemia	MDA-MB435, HL-60		Cu is essential for the cytotoxic activity of elesclomol. Elesclomol selectively transports Cu to the mitochondria, inducing oxidative stress and apoptosis in cancer cells	[86]
Elesclomol/Cu	CRC	SW480, DLD-1		Elesclomol induces Cu-dependent ferroptosis in CRC cells via enhancing the degradation of ATP7A and SLC7A11	[55]

Table 1 (continued)

Compound	Cancer type	Materials (cell lines)	Combined drugs	Anti-tumor effect and its involved mechanism	Refs.
Elesclomol/Cu	Prostate cancer	LNCaP, PC-3, 22RV1	Docetaxel	Elesclomol–Cu suppresses the growth of prostate cancer cells through Cu-induced cuproptosis and enhances their susceptibility to docetaxel chemotherapy both in vitro and in vivo by modulating the DLAT/mTOR pathway	[87]
Elesclomol/Cu	Liver cancer	MHCC-97H, QBC939	Erastin, sorafenib	Elesclomol–Cu induced cuproptosis is enhanced by erastin and sorafenib in liver cancer	[62]
Disulfiram	Pituitary tumor	Primary samples, MMQ cell		Disulfiram can induce cuproptosis in pituitary tumor cells	[88]
Disulfiram	Prostate cancer	PC-3, DU-145		Disulfiram can inhibit the catalytical activity of DNA methyltransferase 1, reduce global 5-methyl cytosine content, reactivate epigenetically silenced genes, and significantly inhibit the growth of prostate cancer cells	[89]
Disulfiram	CRC	H6305-FU, H630WT, DLD-1, RKOWT	5-FU	Disulfiram inhibits both intrinsic and 5-FU-induced NF-κB activity. It enhances the apoptotic effects of 5-FU on DLD-1 and RKOWT cells and effectively overcomes 5-FU chemoresistance in the 5-FU-resistant H630 cell line	[90]
Disulfiram	Breast cancer	MCF-7, SKB-R3, MDA-MB-435S	Cisplatin	Disulfiram inhibits ALDH activity and reduces stemness in ALDH$^+$ breast cancer stem-like cells, while enhancing the cytotoxic effect of cisplatin in these cells	[91]
Disulfiram	Breast cancer	MCF-7, MDA-MB-231		Disulfiram inhibits TGF-β induced EMT in breast cancer cells by downregulating ERK/NF-κB/Snail pathway	[92]
Disulfiram	Gastric cancer	MKN-45, SGC-7901		Disulfiram inhibits the proliferation, migration, and invasion of gastric cancer cells by regulating the Wnt and NF-κB pathways	[93]
Disulfiram	Melanoma	c81-46A, c81-61, c83-2C, A375		Disulfiram induces apoptosis in human metastatic melanoma cells, which is associated with a decrease in mitochondrial membrane potential and ROS production	[94]
Disulfiram	Liver cancer	HepG2, Hepa1-6		Disulfiram induces cystathionine γ-lyase expression and H$_2$S generation in liver cancer cells, sensitizing them to disulfiram	[95]
Disulfiram	Prostate cancer	VCaP, PC-3	Sunitinib	The disulfiram/sunitinib combination induces apoptosis, reduces androgen receptor protein expression, decreases metastatic characteristics, and promotes epithelial differentiation	[96]

Table 1 (continued)

Compound	Cancer type	Materials (cell lines)	Combined drugs	Anti-tumor effect and its involved mechanism	Refs.
Disulfiram	Lung cancer, breast cancer	LLC, B16		Disulfiram interferes with FROUNT-chemokine receptor interactions, inhibiting macrophage responses. Disulfiram monotherapy reduces tumor progression and decreases macrophage tumor-promoting activity	[97]
Disulfiram	GBM	SF188, BT74, U251, Primary patient isolated cells	TMZ	Disulfiram inhibits the growth of TMZ-resistant GBM cells without affecting normal human astrocytes	[98]
Disulfiram	Breast cancer	BT-549, MDA-MB-231		Disulfiram enhances the effectiveness of anti-PD-1 therapy by modulating PD-L1 expression through the epigenetic reactivation of IRF7 in TNBC cells	[99]
Disulfiram	Ovarian cancer	SKOV3, ES-2, OVCA420, HeyA8	Olaparib, niraparib	The combination of PARPis with disulfiram increase ovarian cancer cell apoptosis via upregulating the expression of DNA damage index gH2AX and enhancing PARP cleavage. Disulfiram in combination with Niraparib suppresses growth of ovarian cancer in vivo	[100]
Disulfiram	Liver cancer	Hep3B		Disulfiram inhibits hypoxia-induced gene expression and HIF activity in HCC cells	[101]
Disulfiram	PDAC	PANC-1, BXPC-3, PAN02	αPD-1, gemcitabine	Disulfiram combined with chemoimmunotherapy significantly suppresses the growth of subcutaneous PDAC allograft tumors in mice and extends their lifespan. This combination elevates the proportion of CD8$^+$ T cells and upregulates multiple cytokines in the tumor microenvironment. Disulfiram activates the STING signaling pathway by inhibiting PARP	[102]
Disulfiram	Cervical cancer	Hela		Disulfiram inhibits tumor growth and metastasis by targeting HSP90A/NDRG1/β-catenin pathway in cervical cancer cells	[103]
Disulfiram	Breast cancer	MCF-7	DOX, hydrazine	The triple therapy comprising DOX, disulfiram, and hydrazine demonstrates a synergistic effect, reducing the required DOX dosage to effectively eradicate both wild-type and DOX-resistant MCF-7 cells, thereby augmenting chemosensitivity to DOX	[104]
Disulfiram	NSCLC	A549, NCI-H1975, PC-9, NCI-H460, HCC827, NCI-H1299, NCI-H1975, NCI-H2228	Cisplatin	Disulfiram reacts with cisplatin to form a new platinum chelate, Pt(DDTC)$_3^+$, which exhibits a stronger anti-NSCLC effect than cisplatin alone	[105]
Disulfiram/Cu	Lung cancer	A549	JQ-1/PX478	The combination of disulfiram–Cu and anti-PD-L1 promotes cuproptosis in A549 cells	[106]
Disulfiram/Cu	Breast cancer	MCF-7, MDA-MB-231		CuET suppresses the tumor growth by inhibiting NPL4/p97 segregase-mediated protein degradation	[107]

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Table 1 (continued)

Compound	Cancer type	Materials (cell lines)	Combined drugs	Anti-tumor effect and its involved mechanism	Refs.
Disulfiram/Cu	Breast cancer	MCF7, MDA-MB-231, T47D	Paclitaxel	Disulfiram–Cu inhibits the growth of breast cancer stem cells and enhances the cytotoxicity of paclitaxel in breast cancer cells, potentially due to simultaneous ROS induction and NF-κB inhibition	[108]
Disulfiram/Cu	GBM	U251MG, U87MG, U373MG	Gemcitabine	Disulfiram–Cu induces apoptosis in GBM cells and enhances the cytotoxic effects of gemcitabine on GBM stem-like cells through ROS production, coupled with ALDH activity inhibition and NF-κB signaling pathway suppression	[109]
Disulfiram/Cu	TNBC	MDA-MB-231 PAC10	Paclitaxel/cisplatin	Disulfiram–Cu inhibits the expression of cancer stem cell markers and reverses paclitaxel and cisplatin resistance in MDA-MB-231 PAC10 cells	[110]
Disulfiram/Cu	Colon cancer	H630GEM, HCT116GEM	Gemcitabine	Disulfiram–Cu inhibits gemcitabine-induced NF-κB activation and potentiates gemcitabine cytotoxicity in drug resistant tumor cells	[111]
Disulfiram/Cu	Breast cancer	MDA-MB-231, MCF10DCIS.com		Disulfiram–Cu induces apoptotic cell death and suppresses the tumor xenografts growth of breast cancer cells by inhibiting proteasome activity	[112]
Disulfiram/Cu	Breast cancer	MDA-MB-231, BT20, MDA-MB-231 PIK3CA H1047R, MDA-MB-231 PIK3CA-E545K		Disulfiram–Cu reduces PTEN protein expression and AKT activation in breast cancer cells. Disulfiram–Cu combined with the PI3K inhibitor LY294002 significantly inhibits the growth of tumor xenografts constructed by MDA-MB-231 cells expressing mutant PIK3CA-H1047R and PIK3CA-E545K	[113]
Disulfiram/Cu	HNSCC	FaDu, Hep2		Disulfiram–Cu induces cell death by producing ROS and aggravating autophagy in a Cu-dependent manner in HNSCC cell lines. Disulfiram–Cu inhibits tumor progression in an HNSCC xenograft model	[114]
Disulfiram/Cu	TNBC	MDA-MB-231, 4T1		The disulfiram–Cu treatment promotes apoptosis in TNBC cells via caspase-3 activation and selectively targets cancer stem cell-like populations. These effects are functionally linked to a notable disruption of the STAT3 signaling pathway	[115]
Disulfiram/Cu	Breast cancer	BT474, SKBR3		Disulfiram–Cu induces apoptosis and eliminates cancer stem-like cells in HER2-positive breast cancer by suppressing HER2/Akt signaling pathway	[116]
Disulfiram/Cu	CRC	RKO, Ht29		Disulfiram–Cu significantly inhibits CRC cell viability, predominantly triggering autophagy over apoptosis through ULK1 upregulation	[117]

Table 1 (continued)

Compound	Cancer type	Materials (cell lines)	Combined drugs	Anti-tumor effect and its involved mechanism	Refs.
Disulfiram/Cu	PDAC	PANC-1, PDAC2, PDAC3, PDAC	Irradiation+5-FU	Disulfiram–Cu preferentially targets pancreatic cancer stem cells when combined with chemoradiation, significantly enhancing the in vivo effectiveness of the standard treatment involving irradiation and 5-FU	[118]
Disulfiram/Cu	NSCLC	A549, NCI-H460, NCI-H1299		Disulfiram–Cu inhibits NSCLC cell proliferation by inducing autophagy and apoptosis	[119]
Disulfiram/Cu	CRC	HCT116, SW620, HCT8		Disulfiram–Cu can suppress the growth of CRC cell, which may be related to the upregulation of ICD	[120]
Disulfiram/Cu	MM, AML, ALL	Primary cells obtained from patients		Disulfiram–Cu induces the loss of mitochondrial membrane potential, triggers the ROS production, and activates executioner caspases	[121]
Disulfiram/Cu	Lymphoid malignances	Raji, Molt4		Disulfiram–Cu suppresses the growth of lymphoid malignant cells via ROS-JNK activation and inhibition of the Nrf2 and NF-κB pathways	[122]
Disulfiram/Cu	CRC	HCT116		Disulfiram reduces intracellular H_2S levels and inhibits the proliferation of HCT116 cells through its metabolite CuDDC	[123]
Disulfiram/Cu	GBM	Patient-derived BTIC	TMZ	Disulfiram–Cu significantly enhances TMZ activity in vitro and prolongs survival in vivo in patient-derived BTIC models by inhibiting proteasome activity and functionally impairing DNA repair pathways	[124]
Disulfiram/cadmium (Cd)	Breast cancer	MCF10DCIS, MCF10A		Disulfiram–Cd selectively induces proteasome dysfunction and apoptosis in human breast cancer cells, while sparing non-tumorigenic cells	[125]
Disulfiram/Cu	Breast cancer	MDA-MB-231, Hs578T		Disulfiram–Cu exerts anti-migratory and anti-invasive effects by inducing focal adhesion loss and cytoskeletal collapse, thereby suppressing tumor growth and lung colonization in TNBC	[126]
Disulfiram/Cu	Medulloblastoma	UW228, D341, D425		Disulfiram–Cu induces cell death by causing NPL4 accumulation in the cell nucleus and the buildup of polyubiquitinated proteins. In vivo, disulfiram–Cu prolongs survival and increases nuclear NPL4 expression	[127]
Disulfiram/Cu	Chondrosarcoma	SW1353, CS-1	Irradiation	Disulfiram–Cu radiosensitizes chondrosarcoma stem cells in vitro and in vivo	[128]
Disulfiram/Cu	Cervical cancer	SiHa, HeLa		Disulfiram–Cu induces deregulation of the S-phase, inhibits the expression of stemness markers, and reduces the population of cancer stem cell-like LGR5+ cells	[129]

Table 1 (continued)

Compound	Cancer type	Materials (cell lines)	Combined drugs	Anti-tumor effect and its involved mechanism	Refs.
Disulfiram/Cu	Thyroid carcinoma	K1 (A), WRO		Disulfiram–Cu suppresses cancer stem cell activity in differentiated thyroid cancer cells by downregulating c-Myc/E2F1-mediated BMI1 expression	[130]
Disulfiram/Cu	Ovarian cancer	OVCAR3, SKOV-3		Disulfiram–Cu induces apoptosis and activates heat shock proteins in ovarian cancer cells. Short-term disulfiram–Cu treatment causes irreversible cell damage, characterized by cell detachment and actin fiber depolymerization	[131]
Disulfiram/Cu	Breast cancer, colon cancer	MCF-7, HT-29		Acidic pH significantly enhances the toxicity of the disulfiram–Cu complex in breast and colon cancer cells. This effect is associated with changes in cell metabolism, altered Akt kinase and NF-κB activity, and increased ROS production	[132]
Disulfiram/Cu	HCC	HCC-LM3, Huh7, Hepa1-6	anti-CD47 antibody	Disulfiram–Cu induces ICD-mediated immune activation in HCC and enhances the efficacy of CD47 blockade. Mechanistically, disulfiram–Cu promotes the nuclear accumulation and aggregation of NPL4, inhibiting the ubiquitin–proteasome system and inducing ER stress	[133]
Disulfiram/Cu	Thyroid cancer	8305C, 8505C, BCPAP, IHH4	Vemurafenib	Disulfiram–Cu kills BRAF-mutant thyroid cancer cells and sensitizes them to BRAF kinase inhibitors by relieving feedback activation of the MAPK/ERK and PI3K/AKT pathways in a ROS-dependent manner	[134]
Disulfiram/Cu	HCC	Hep3B, Hepa1-6	αPD-1	Disulfiram–Cu fails to inhibit HCC tumor growth in C57BL/6 mice. However, disulfiram–Cu can restrain GSK3β activity by inhibiting PARP1, resulting in the upregulation of PD-L1 expression. Combination therapy with disulfiram–Cu and αPD-1 demonstrated significantly better antitumor efficacy than monotherapy	[135]
Disulfiram/Cu	HCC	Huh7, SMMC-7721	sorafenib	Disulfiram–Cu severely disrupts mitochondrial homeostasis, increases the free iron pool, enhances lipid peroxidation, and ultimately induces ferroptotic cell death. By simultaneously inhibiting the NRF2 and MAPK kinase signaling pathways, disulfiram–Cu enhances the cytotoxicity of sorafenib and arrests tumor growth both in vitro and in vivo	[136]
Disulfiram/Cu	NSCLC	H522, H2030, A549, H1975	Sulfasalazine	Disulfiram–Cu can abrogate sulfasalazine-induced expression of NRF2, SLC7A11, and ALDH1A1. The combination of sulfasalazine and disulfiram–Cu can induce significantly higher cytotoxic effects and suppress the malignant progression of NNK-induced lung tumors	[137]

Table 1 (continued)

Compound	Cancer type	Materials (cell lines)	Combined drugs	Anti-tumor effect and its involved mechanism	Refs.
Disulfiram/Cu	DS-AMKL	Primary patient isolated cells	BTZ, Ara-C	Disulfiram–Cu overcomes resistance to Ara-C and BTZ in cell lines derived from DS-AMKL patients	[138]
Disulfiram/Cu	Metastatic prostate cancer	mPCa cell line	Docetaxel	Disulfiram–Cu significantly enhances the antitumor effects of docetaxel in a docetaxel-resistant xenograft tumor model by downregulating the expression of ATP7B	[139]
ATSM	Prostate cancer	PC3, Du145, LNCaP, TRAMP		ATSM-Cu triggers cytotoxicity in human prostate hyperplastic and carcinoma cell lines while leaving primary prostate epithelial cells unaffected	[140]
GTSM	Prostate cancer	PC3, Du145, LNCaP, TRAMP		GTSM-Cu triggers cytotoxicity in human prostate hyperplastic and carcinoma cell lines while leaving primary prostate epithelial cells unaffected	[140]
CQ	Prostate cancer	PC3, Du145, LNCaP		CQ-Cu promotes apoptosis by facilitating the degradation of XIAP in cancerous prostate cells	[141]
Curcumin	CRC	Caco-2		Curcumin regulates lipid, RNA, NADH, and NADPH metabolism, upregulates positive cuproptosis mediators, and enhances cuproptosis in CRC cells	[142, 143]
SIH	Liver cancer	HepG2		SIH facilitates the transport and intracellular release of Cu^{2+} in HepG2 cells, thereby triggering mitochondria-mediated apoptosis	[144]

ES elesclomol, *DSF* disulfiram, *ROS* reactive oxygen species, *DOX* doxorubicin, *GBM* glioblastoma, *GSCs* GBM stem-like cells, *GdECs* GSC-derived endothelial cells, *TMZ* temozolomide, *ARID1A* AT-rich interactive domain 1A, *BRCA1* breast cancer susceptibility gene 1, *CRC* colorectal cancer, *TGF-β* transforming growth factor-β, *EMT* epithelial-mesenchymal transition, *PDAC* pancreatic ductal adenocarcinoma, *5-FU* 5-fluorouracil, *HCC* hepatocellular carcinoma, *HIF* hypoxia inducible factor, *PD-L1* programmed death-ligand 1, *PARP* poly ADP-ribose polymerase, *CuET* bis-diethyldithiocarbamate-Cu, *PTEN* Phosphatase and tensin homolog deleted on chromosome ten, *HNSCC* head and neck squamous cell carcinoma, *TNBC* triple negative breast cancer, *HER2* human epidermal growth factor receptor 2, *ULK1* Unc-51-like autophagy activating kinase 1, *NSCLC* non-small cell lung cancer, *MM* multiple myeloma, *AML* acute myeloid, *ALL* lymphoblastic leukemia, *ICD* immunogenic cell death, *JNK* Jun N-terminal kinase, *BTIC* brain tumor-initiating cell, *LGR5* leucine-rich repeat-containing G protein-coupled receptor 5, *SLC7A11* solute carrier family 7 membrane 11, *Nrf2* nuclear factor erythroid-2-related factor 2, *NNK* 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, *DS-AMKL* down-syndrome-associated acute myeloid leukemia, *BTZ* bortezomib, *Ara-C* cytarabine, *NADH* nicotinamide adenine dinucleotide, *NADPH* nicotinamide adenine dinucleotide phosphate, *ATSM* diacetyl-bis (N4-methylthiosemicarbazone), *TRAMP* transgenic adenocarcinoma of mouse prostate, *GTSM* glyoxal-bis (N4-methylthiosemicarbazone), *CQ* 7-iodo-5-chloro-8-hydroxyquinoline, *SIH* salicylaldehyde isonicotinoyl hydrazone

requires further investigation. The crosstalk mechanisms between cuproptosis and other forms of cell death are poorly understood and necessitate more research to unveil, possibly facilitating the development of effective anticancer strategies based on elesclomol–Cu. Elesclomol has not yet shown effective therapeutic outcomes in clinical trials [148]. A possible reason is that single-agent elesclomol treatment may not elevate Cu levels in tumor cells to those required to trigger cuproptosis. A subsequent phase III trial results revealed that although the combination of elesclomol with paclitaxel did not improve progression-free survival in melanoma patients, elesclomol exhibited better anti-tumor effects in patients with low lactate dehydrogenase (LDH) levels [149]. Low LDH represents diminished glycolysis [150], reflecting enhanced mitochondrial metabolism. This aligns with Tsvetkov's finding that cuproptosis relies on mitochondrial metabolism.

Disulfiram is an aldehyde dehydrogenase (ALDH) inhibitor FDA-approved for treating alcoholism. Disulfiram has also been deeply studied for an extended period in anti-tumor research (Table 1). Disulfiram interacts with Cu as a Cu ionophore to form the metabolite bis-diethyldithiocarbamate-Cu (CuET), which transports Cu across the cell membrane [151]. Disulfiram–Cu–induced cellular damage is also linked to apoptosis, ferroptosis, and cuproptosis (Table 1). Multiple targets or signaling pathways have been reported to be associated with the anti-tumor activity of disulfiram–Cu, such as ROS levels [94, 108, 109, 121, 122, 132, 134, 137], the ubiquitin–proteasome system [112, 124, 125, 133], the JNK and p38 pathways [66, 67, 114, 122], the NF-kB pathway [90, 92, 93, 109, 111, 122], and NPL4 [107, 127, 133]. In addition, disulfiram–Cu has been reported to overcome tumor drug resistance to cisplatin [91, 110], paclitaxel [108, 110], gemcitabine [109, 111], 5-fluorouracil (5-FU) [90, 118], temozolomide [98, 124], and sunitinib [96]. Table 1 summarizes the anti-tumor function of disulfiram in preclinical studies. Although, like elesclomol, disulfiram has presented significant anti-tumor effects in preclinical experiments, exciting results have yet to emerge from clinical trials [152, 153]. One limiting factor may be the inability to maintain high Cu levels in patient tumor cells. However, given the good clinical safety profile, conducting more clinical trials that combine elesclomol or disulfiram with clinical drugs could facilitate the translation of cuproptosis-associated anti-tumor therapies from the laboratory to clinical practice.

Besides elesclomol and disulfiram, other compounds, such as diacetyl-bis (N4-methylthiosemicarbazone) (ATSM) and glyoxal-bis (N4-methylthiosemicarbazone) (GTSM), have been identified as Cu ionophores (Table 1). Cu complexes with ATSM or GTSM induced cytotoxicity in human prostate hyperplastic and carcinoma cell lines without affecting the primary prostate epithelial cells [140]. This selective cytotoxicity may be associated with differential Cu levels in tumor cells, as Cu concentrations are elevated in prostate cancer cells compared to normal prostate epithelial cells [154]. Additionally, 7-iodo-5-chloro-8-hydroxyquinoline (CQ) has been reported to mediate Cu accumulation in cells [141]. In cancerous prostate cells, rather than normal prostate cells, CQ-Cu complexes promote apoptosis by facilitating the degradation of XIAP, a protein that inhibits caspases [141]. Notably, CQ can induce cellular damage via multiple pathways, including proteasome and lysosome dysfunction, conferring severe toxic side effects that limit its clinical application in cancer therapy [155]. Recent studies have also revealed that curcumin, a natural compound derived from *Curcuma longa*, can act as a Cu ionophore and promote cuproptosis in CRC cells [142, 143]. Curcumin is a potential anticancer natural product that can inhibit the cell cycle, induce apoptosis, and activate tumor suppressors. Additionally, several clinical studies have shown that curcumin has good efficacy and safety [156]. These characteristics make curcumin a promising cuproptosis inducer for clinical application. Furthermore, salicylaldehyde isonicotinoyl hydrazone (SIH), a lipophilic tridentate iron chelator, can facilitate the transportation and intracellular release of Cu^{2+} in HepG2 cells, thereby triggering mitochondria-mediated apoptosis, suggesting that SIH is also a Cu ionophore [144]. However, the cell death induced by these Cu ionophores, beyond elesclomol and disulfiram, whether related to the aggregation of lipoylated proteins and the reduction of Fe–S proteins, requires further investigation.

During cuproptosis, the primary function of Cu ionophores is to transport Cu across the cell membrane and release it into the cell. Notably, most metal-ion ionophores are not specific to a single metal element. For instance, elesclomol can directly bind Fe^{2+} [157]. Besides, elesclomol and disulfiram can increase cellular iron content during transporting Cu into cells [158, 159]. This non-specificity complicates the mechanism of cell damage caused by Cu ionophores combined with Cu. However, treatment with such agents can cause metal dyshomeostasis, exacerbating the side effects of the therapy. Consequently, developing Cu- or tumor-specific ionophores represents a potential breakthrough in accelerating the clinical application of Cu ionophores for cancer treatment.

Small compounds

Cu is a dual-faceted player in tumorigenesis. A high Cu level promotes tumor cell proliferation and growth, suggesting some resistance mechanisms to cuproptosis

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in tumor cells. As a result, small-molecule compounds that disrupt Cu homeostasis may also induce or increase the sensitivity of tumor cells to cuproptosis. Recent studies have unveiled several small compounds capable of inducing cuproptosis (Table 2). For instance, Yang et al. discovered that zinc pyrithione can induce cuproptosis in triple negative breast cancer (TNBC) cells by disrupting intracellular Cu homeostasis and DLAT oligomerization, potentially contributing to the chemosensitivity of TNBC [160]. In CRC cells, 4-Octyl itaconate (4-OI) inhibits glycolysis by targeting the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), promoting elesclomol–Cu-mediated cuproptosis [161]. Besides, anisomycin, a well-known inhibitor of protein synthesis that binds to the 60S ribosomal subunits, has been found to bind and inhibit Yinyang 1 (YY1), inactivating the transcriptional activity of core genes of the LA pathway (*FDX1*, *DLD*, *DLAT*, and *PDHB*), potentially leading to cuproptosis in ovarian cancer stem cells [162]. Additionally, as mentioned above, sorafenib, the first multi-tyrosine kinase inhibitor approved for treating many cancers and capable of inducing ferroptosis, and erastin, a commonly used ferroptosis inducer, can aggravate cuproptosis in liver cancer cells [62]. Compared to Cu ionophores, small molecular compounds that disrupt tumor cell Cu homeostasis can induce tumor cell cuproptosis without Cu supplementation, thereby avoiding the imbalance of metal ions in the body and reducing metal-induced side effects during treatment. The cuproptosis research is currently in its infancy. The development and discovery of more cuproptosis inducers, especially those based on drugs approved for clinical use, could significantly promote the clinical application of cuproptosis-targeted cancer treatment strategies.

Nanomedicine

Dissolving, adsorption, encapsulation, or attachment to nanomatrices can transform drugs into nanomedicines. These nanomedicines use the characteristics of tumor tissues or cells, such as acidic environments, elevated GSH and ROS levels, and tumor cell-specific surface markers, to accumulate or release within tumor sites. This strategic approach can increase the precision of drug delivery and minimize the side effects of cancer therapy. Given the relatively low selectivity of Cu ionophores toward tumor cells, using a nanoparticle-based delivery system for the precise delivery of Cu to tumor cells can effectively enhance cuproptosis in tumor tissues while reducing damage to other normal tissues. Since cuproptosis was identified, more studies have focused on this area (Table 3). For instance, DSF@PEG/copper-HMSNs can precisely release Cu^{2+} and disulfiram in tumor tissues to induce cuproptosis and inhibit tumor growth [166]. Au NCs-Cu^{2+}@SA-HA NHGs can enhance cuproptosis-mediated tumor therapy by depleting GSH and H_2O_2 in the tumor tissues [180].

Researchers have aimed to precisely deliver Cu, Cu ionophores, and other anticancer agents, such as chemotherapeutic drugs and siRNA (Table 3), to explore tumor therapy strategies based on nanomedicine-induced cuproptosis. This approach enhances cell damage through other mechanisms or sensitization to cuproptosis, thereby synergistically combating tumors. For example, TP-M–Cu–MOF/siATP7a efficiently silences the *ATP7A* gene and increases Cu intake, thus inducing cuproptosis and enhancing anti-tumor efficacy [178]. OMP contains siRNA targeting *PDK1*, which, during releasing Cu^{2+}, can reduce cellular glycolysis by decreasing PDK1 expression, thereby sensitizing cells to cuproptosis [186]. LDH/HA/5-FU nanosheets can release 5-FU

Table 2 Small compounds capable of inducing cuproptosis

Compound	Cancer type	Materials (cells)	The effect on cuproptosis and its involved mechanism	Refs.
ZnPT	TNBC	MDA-MB-231, HCC1806	ZnPT induces cuproptosis by disrupting intracellular copper homeostasis and DLAT oligomerization. ZnPT-induced cuproptosis potentially contributes to chemosensitivity of TNBC	[160]
4-OI	CRC	HCT116, LoVo	4-OI inhibits glycolysis by targeting GAPDH to promote elesclomol–Cu-mediated cuproptosis	[161]
Anisomycin	Ovarian cancer	Human ovarian cancer stem cells	Anisomycin has a potential toxicity of promoting cuproptosis in human ovarian cancer stem cells by attenuating YY1/lipoic acid pathway activation	[162]
Sorafenib	HCC, ICC	MHCC-97H, Huh7, QBC939, CCLP1	Sorafenib enhances cuproptosis in HCC cells by increasing Cu-dependent lipoylated protein aggregation and reducing intracellular GSH synthesis	[62]
Erastin	HCC, ICC	MHCC-97H, Huh7, QBC939, CCLP1	Erastin enhances cuproptosis in HCC cells by increasing Cu-dependent lipoylated protein aggregation and reducing intracellular GSH synthesis	[62]
IKE	MDS	SKM-1, MUTZ-1	IKE synergistically enhances elesclomol–Cu-mediated cytotoxicity in MDS cell lines by triggering cuproptosis and ferroptosis	[163]

ZnPT zinc pyrithione, *TNBC* triple negative breast cancer, *4-OI* 4-Octyl itaconate, *CRC* colorectal cancer, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *HCC* hepatocellular carcinoma, *ICC* intrahepatic cholangiocarcinoma, *GSH* glutathione, *MDS* myelodysplastic syndromes, *IKE* imidazole ketone erastin

Table 3 Nanomedicines targeting cuproptosis for cancer treatment

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
GOx@[Cu(tz)]	GOx, Cu_2O, 1,2,4-triazole	Bladder cancer	5637 tumor	PDT		GOx@[Cu(tz)] induces cuproptosis in cancer cells under conditions of glucose depletion and suppresses tumor growth of 5637 bladder tumorsin in athymic	[164]
$Cu_2(PO_4)(OH)$ NPs	$Cu_2(PO_4)(OH)$	CRC	HCT116 tumor			$Cu_2(PO_4)(OH)$ NPs can induce pyroptosis by ROS-mediated Caspase 1 activation and gasdermin D cleavage, and efficiently induce cuproptosis by downregulating ATP7A to maximizing Cu overload	[165]
DSF@PEG/Cu-HMSNs	Disulfiram, PEG, Cu^{2+}, HMSNs	Breast cancer	4T1 tumor	PTT		DSF@PEG/Cu-HMSNs can induce toxic mitochondrial protein aggregation, leading to cell cuproptosis	[166]
HFn-Cu-REGO NPs	Human heavy chain ferritin, Cu^{2+}, regorafenib	GBM	U251, U87 tumor		regorafenib	HFn-Cu-REGO NPs could result in lethal autophagy arrest in GBM cells via releasing regorafenib that can inhibit autophagosome-lysosome fusion, and disturbs copper homeostasis for triggering cuproptosis by releasing Cu^{2+}	[167]
Cu-GA NPs	Cu^{2+}, Gallic acid, polyvinylpyrrolidone	Breast cancer	4T1 tumor	CDT		Cu-GA NPs can induce severe cell cuproptosis and apoptosis by depleting intracellullar GSH ad generating ROS, and effectively suppress tumor growth when combining with CDT	[168]
Au@MSN-Cu/PEG/DSF	Au nanorods, $Cu(NO_3)_2$, PEG, disulfiram	Breast cancer	4T1 tumor	PTT		In synergy with PTT, Au@MSN-Cu/PEG/DSF could effectively kill tumor cells and inhibit tumor growth by inducing the cell apoptosis and cuproptosis	[169]
DMMA@Cu_{2-x}Se	Poly(ethylene imine), 2,3-dimethylmaleic anhydride, Cu_{2-x}Se, RGD polypeptide	Melanoma	A375 tumor	PTT		DMMA@Cu_{2-x}Se can induce cuproptosis by releasing Cu^{2+}, which further improves thermotherapy by up-regulating mitochondrial damage-meidated ROS. DMMA@Cu_{2-x}Se combined with laser has a satisfactory antitumor effect in melanoma tumor-bearing nude mice	[170]

Table 3 (continued)

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
NP@ESCu	Amphiphilic biodegradable polymer, elesclomol–Cu	Bladder cancer	MB49 tumor		αPD-L1	NP@ESCu could induce tumor cell cuproptosis and reprograme TME. Combined with αPD-L1, NP@ESCu can efficiently suppress tumor growth in mouse models with subcutaneous bladder cancer	[171]
HNP	DTPH, Cu^{2+}, disulfiram, hyaluronan, artemisinin	Breast cancer	4T1 tumor			HNP could deplete GSH by riched disulfide bonds to sensitize cancer cells to the cuproptosis. HNP can effectively suppress tumor growth by a synergistic combination of cuproptosis, ferroptosis, and apoptosis	[172]
CS/MTO-Cu@AMI	Mitoxantrone, Cu^{2+}, amiloride, chondroitin sulfate	Breast cancer	4T1 tumor			CS/MTO-Cu@AMI induces cuproptosis and mitochondrial damage, which actviates the AMPK pathway to orchestrate PD-L1 degradation. CS/MTO-Cu@AMI could induce anti-tumor immunity by activating the cGAS-STING pathway	[173]
CCJD-FA	CaO_2, Cu_{2+}, DTPH, DSPE-PEG-FA, JQ-1	CRC	CT26 tumor		JQ-1	CCJD-FA could induce severe cuproptosis by releasing Cu and inhibiting intracellular glycolysis and ATP production, and reduce the expression of IFN-γ-induced PD-L1 by suppressing BRD4. These effects make cancer cells more susceptible to cuproptosis and reshape immunosuppressive TME to inhibit tumor growth	[174]
CuET NPs	BSA, CuET, NaDTC	Lung cancer	A549 tumor			CuET NPs can suppress the growth of cisplatin-resistant tumor cells in vitro and in vivo with superior biosafety	[175]
BSO-CAT@MOF-199 @DDM (BCMD)	BSO, DDM, Cu-based MOF of MOF-199	GBM	GL261 tumor		αPD-L1	BCMD could induce tumor cell cuproptsis, which triggers ICD and enhances tumoricidal immunity. Combining with αPD-L1, BCMD could further cooperate to reconstruct the TIME and enhance the therapeutic effect	[176]

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Table 3 (continued)

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
CuMoO$_4$ Nanodots	Cu^{2+}, MoO$_4^{2-}$, SDS	Breast cancer	MCF-7, 4T1 tumor	PTT		Under sustained PTT, CuMoO$_4$ Nanodots can effectively induce ferroptosis and cuproptosis in tumor cells and trigger an immune response to ICD	[177]
TP–M–Cu–MOF/siATP7a	Cu–MOF, siATP7a, TPM	HSCLC	H69 tumor			TP–M–Cu–MOF/siATP7a presents high blood–brain barrier transcytosis and specific uptake by tumor cells within the brain, and exhibits high silencing efficiency against ATP7A to increasing copper intake, thereby inducing cuproptosis and enhancing therapeutic efficacy in HSCLC brain metastasis tumor-bearing mice	[178]
SonoCu	Cu^{2+}, zeolitic imidazolate framework-8, perfluorocarbon, chlorin e6, O$_2$	Breast cancer	4T1 tumor	SDT		Combining with SDT, SonoCu can induce tumor cell cuproptosis but sparing normal cells. SonoCu provides a desirable antitumor outcome with good biosafety	[179]
Au NCs-Cu^{2+}@SA-HA NHGs	NAC, 4-mercaptobenzoic acid, HAuCl$_4$, NaOH, NaBH$_4$, CuCl$_2$,	HCC	HepG2, H22 tumor	PTT, PDT		Au NCs-Cu^{2+}@SA-HA NHGs can respond to NIR, enhancing PTT and PDT. The release of Cu^{2+} triggers cuproptosis, catalyzes H$_2$O$_2$ to generate O$_2$, and consumes GSH to form hydroxyl radicals, synergistically improving PDT and CDT	[180]
CuX-P	PD-1 overexpressing T cell membrane, Mxene, Cu^{2+}, disulfiram	Breast cancer	4T1 tumor	PTT		CuX-P can bind to PD-L1 on tumor cells, leading to internalization and upregulation of PD-L1 expression. The feedback loop between CuX-P and PD-L1 promotes PD-L1 consumption and CuX-P enrichment in tumors, inducing cuproptosis. Laser treatment with CuX-P stimulates potent anti-tumor immune responses	[181]
Cu2O@CuBTC-DSF@HA nanocomposites (CCDHs)	Cu$_2$O, Trimesic acid, disulfiram, Hyaluronic acid	Breast cancer	4T1 tumor			CCDHs synergistically enhance cuproptosis instead of inducing apoptosis, demonstrating superior anti-tumor efficacy with minimal toxicity	[182]

Table 3 (continued)

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
Cu-LDH	Layered double hydroxide, Cu^{2+}	Breast cancer	4T1 tumor			Cu-LDH nanoparticles function as lysosome destroyers, enhancing Cu overload-mediated cuproptosis and pyroptosis for efficient cancer immunotherapy	[183]
PCM nanoinducers	PEG-polyphenol-Ce6 polymer; Cu^{2+}; Mdivi-1;	Breast cancer	4T1 tumor		Mdivi-1	PCM nanoinducers amplify proteotoxic stress through cuproptosis and cause mtDNA release, activating the cGAS-STING pathway. This activation triggers both innate and adaptive immune responses, effectively combating tumor growth and systemic metastasis	[184]
Cu-DBCO/CL	Cu-DBCO; CHO; LOX-IN-3; 2,2'-PSDA	Breast cancer	4T1 tumor			Cu-DBCO/CL induces tumor cell cuproptosis and ferroptosis, simultaneously enhancing ICD and remodeling the ECM, resulting in significant tumor growth and metastasis inhibition	[185]
OMP	OPDEA, 2-methylimidazole, $Cu(NO_3)_2$, $Zn(NO_3)_2·6H_2O$, siPDK	Melanoma	Mice-bearing B16F10 lung melanoma metastasis		aPD-L1	OMP induces tumor cells cuproptosis. siPDK released from OMP sensitizes the cuproptosis by inhibiting intracellular glycolysis and ATP production, and blocking the Cu^+ efflux protein ATP7B. OMP-mediated cuproptosis triggers ICD to promote DCs maturation and $CD8^+$ T cells infiltration, and upregulates membrane-associated PD-L1 expression, offering improved efficacy against lung metastasis when combined with aPD-L1	[186]
M@HMnO₂-DP	$HMnO_2$, disulfiram, Prodrugs 4 and 5, 4T1 cancer cell membrane	Breast cancer	4T1 tumor			M@HMnO₂-DP specifically targets cancer cells to deliver disulfiram, triggering cuproptosis without exogenous Cu. This compound inhibits tumor cell glycolysis through bioorthogonal chemistry-based drug synthesis and disrupts Fe–S protein biosynthesis, enhancing cuproptosis sensitivity	[187]

Table 3 (continued)

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
PDA-DTC/Cu NPs	Diethyldithiocarbamate, Polydopamine, Cu^{2+}	Breast cancer	4T1 tumor			PDA-DTC/Cu NPs induce the tumor cell cuproptosis by elevating intracellular Cu accumulation, disrupting mitochondrial function, and restricting the ATP energy supply, promoting the repolarization of TAMs to relieve the TIME	[188]
HA-CD@MOF NPs	Cu^{2+}, DOX, hyaluronate acid	Breast cancer	4T1 tumor	CDT	DOX	Combining chemodynamic therapy with Cu^{2+} overload exacerbates ROS storms and mitochondrial damage, sensitizing cuproptosis. HA-CD@MOF NPs robustly activate ICD and suppress tumor metastasis	[189]
MCD	MSN, Cu_2S; oxidized dextran	Osteosarcoma	[143B tumor	PTT		MCD triggers tumor cell cuproptosis by inhibiting key TCA cycle proteins, showing promising mild-temperature PTT in the NIR-II range, effectively reducing tumor growth and OS-induced bone destruction in vivo	[190]
CDPh	DMONs, Cu, phloretin	CRC	CT26 tumor	PTT		In-situ activation of CDph facilitates Cu dissociation and glutathione consumption, triggering cuproptosis. The ROS storm generated by mild photothermal-enhanced peroxidase (POD)-like reaction and glycolysis interference by glucose transporter inhibitor phloretin (Ph) synergistically disrupts cellular Cu homeostasis, leading to cancer cell cuproptosis	[191]
CuET@PH NPs	polydopamine; hydroxyethyl starch; CuET	PDAC	Panc02 tumor	HBO		HBO and CuET@PH NPs inhibit glycolysis and oxidative phosphorylation, respectively, suppressing cancer stem cell energy metabolism, achieving robust tumor inhibition in PDAC and significantly extending mouse survival	[192]

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Table 3 (continued)

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
CuO$_2$/DDP@SiO$_2$	CuO$_2$; DDP; SiO$_2$	HCC	H22 tumor	CDT	Cisplatin	CuO$_2$ generates O$_2$, increases pH, and oxidizes intracellular GSH, sensitizing cancer cells to CuO2/DDP@SiO2-mediated cuproptosis. CuO$_2$ significantly downregulates multi-drug resistance-associated protein 2 through O$_2$-dependent HIF-1 inactivation, blocking the cisplatin-efflux pathway and enhancing cisplatin's antitumor effects	[193]
$^{Cu/AP}$H-M	HPB, Au–Pt nanozymes, Cu^{2+}, cancer cell membrane;	CRC	CT26 tumor		αPD-L1	Cu/APH-M effectively carries Cu and induces cuproptosis in tumor cells. Cu/APH-M also can enhance the oxygenation of the TME to trigger robust antitumor immunity, and synergize with immuno-therapy to prevent distant tumor recurrence, particularly in low rectal cancer	[194]
PCD@CM	NIR-II ultrasmall polymer dots; Cu^{2+}; DOX; 4T1 cell membrane	Breast cancer	4T1 tumor		DOX, aPD-L1	PCD@CM induces the aggregation of lipoylated mitochondrial proteins and the loss of iron-sulfur proteins, leading to severe proteotoxic stress and ultimately cuproptosis. NIR-II PTT and GSH depletion render tumor cells more sensitive to cuproptosis. The amplified cuproptosis triggers ICD to promote cytotoxic T lymphocyte infiltration along with aPD-L1-mediated immune checkpoint blockade	[195]
ES@CuO	CuO; elesclomol; glycol polymer	Melanoma	B16 tumor		αPD-1	ES@CuO synergistically triggers tumor cell cuproptosis, promotes cuproptosis-mediated immune responses, and increases the number of tumor-infiltrating lymphocytes and secreted inflammatory cytokines. Combining ES@CuO with PD-1 immunotherapy substantially increases antitumor efficacy in murine melanoma	[196]

Table 3 (continued)

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
D-CuxOS@Fe–MOF	Cu^{2+}; Fe^{3+}; D-/L-penicillamine; NH2-BDC	Breast cancer	4T1 tumor			D-CuxOS@Fe–MOF induces augmented oxidative stress and potent ferroptosis, synergizing with cuproptosis for enhanced cancer therapy	[197]
ES-Cu-Alg hydrogel	Cu^{2+}; elesclomol; sodium alginate; galactose	CRC	CT26 tumor			Elesclomol–Cu–Alg hydrogel effectively induces cuproptosis in colorectal cancer cells, abrogates radiation-induced PD-L1 upregulation, and sensitizes tumor cells to radiotherapy and immunotherapy	[198]
T-HCN@CuMS	HCN, CuMS, cRGDfk-PEG2k-DSPE	Osteosarcoma	[143B tumor	PTT		T-HCN@CuMS demonstrates favorable photo-induced catalytic properties, generating abundant ROS under NIR light irradiation. It efficiently catalyzes the Fenton-like reaction, triggering cell cuproptosis and achieving favorable therapeutic outcomes to inhibit tumor growth and metastasis	[199]
CJS-Cu NPs	BETA, Cu^+	Breast cancer	4T1 tumor	PTT		CJS-Cu NPs selectively induce cuproptosis and downregulate metastasis-related protein expression, contributing to the complete inhibition of lung metastasis	[200]
CQG NPs	Cu^{2+}; Polyvinylpyrrolidone, Gallic acid; (3-aminopropyl) triethoxysilane, GOx	Breast cancer	4T1 tumor			CQG NPs induce cuproptosis by releasing Cu and depleting endogenous Cu chelators, disrupting the antioxidant defense mechanism of tumor cells. This promotes immunosuppressive TME remodeling, enhances immune cell infiltration into the tumor, and activates robust systemic immunity	[201]
Cu₂O@Mn₃Cu₃O₈ (CMCO) nanozyme	Cu_2O; $Mn_3Cu_3O_8$	CRC	CT26 tumor	PTT		CMCO nanozyme induces high-efficiency ferroptosis-boosted cuproptosis via a mild-photothermal effect for colorectal cancer therapy	[202]

Table 3 (continued)

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
Cu-doped BiSex (CBS)	CuI; Bi$_2$Se$_3$		PC-3 tumor	PTT	αPD-L1	CBS induces cuproptosis and apoptosis through photothermal effects and augmented oxidative stress, boosting antitumor immune responses when combined with αPD-L1	[203]
E-C@DOX NPs	Cu^{2+}; ellagic acid, DOX, chondroitin sulfate	Breast cancer	4T1 tumor; MCF7^{Adr} tumor		DOX	E-C@DOX NPs inhibit tumor cell stemness and cell survival-related pathways while working in tandem with Cu to damage mitochondria and induce cuproptosis. This suppresses the ATP-dependent drug efflux pathway, reversing DOX resistance	[204]
E. coli@Cu$_2$O	E. coli; Cu$_2$O	Colon tumor	MC 38 tumor	PTT	αPD-1	E. coli@Cu$_2$O induces celllular ferroptosis and cuproptosis. Photothermal-enhanced ferroptosis/cuproptosis reverses the immunosuppression of colon tumors by triggering DC maturation and T cell activation	[205]
Cu@CDCN	Cu^{2+}, carbon photocatalyst	Breast cancer	4T1 tumor	PTT		Cu@CDCN, with efficient photocatalytic H$_2$ production and anchored Cu^{2+}, enables a combination of hydrogen therapy and cuproptosis, causing mitochondrial damage and inhibiting tumor growth	[206]
LDH/HA/5-FU nanosheets	5-FU; copper–aluminum layered double hydroxide, hyaluronic acid	Breast cancer	4T1 tumor	CDT	5-FU	LDH/HA/5-FU nanosheets specifically target tumor cells, rapidly release Cu^{2+} and 5-FU, and induce tumor cell apoptosis and cuproptosis. These nanosheets successfully promote the immune system, combining Cu-based CDT and chemotherapy, showing promising potential for solid tumor treatments	[207]

Table 3 (continued)

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
PEG@Cu$_2$O-ES	Cu$_2$O; Elesclomol; PEG	Breast cancer	4T1 tumor	PTT, CDT	αPD-1	PEG@Cu$_2$O-ES, with PTT and CDT effects, generates ROS to attack the ATP-Cu pump, reducing Cu ion outflow and aggravating cuproptosis. PEG@Cu$_2$O-ES shows strong antitumor effects by inducing cuproptosis, reprogramming TME, and increasing response sensitivity to αPD-1	[208]
CCNAs	ZnPc; thioketal; 1-MT; DOX; Cu^{2+}	Prostate cancer	PC-3 tumor	PTT	DOX	Upon NIR laser irradiation, ZnPc exhibits a photodynamic effect generating ROS, triggering DOX release, and enhancing tumor cell apoptosis. Cu^{2+} in the CCNAs enhances the photodynamic process, promoting toxic mitochondrial protein aggregation and leading to cell cuproptosis. This intensified cuproptosis-apoptosis effect triggers an ICD response, and released 1-MT reverses ITM by suppressing IDO-1-mediated Trp degradation	[209]
ZCProP	Zeolitic imidazole framework-90, Cu^{2+}, prodigiosin, PEG	Breast cancer	4T1 tumor			ZCProP delivers Cu and prodigiosin to mitochondria, inducing cell death through synergistic mechanisms of cuproptosis, ferroptosis, and apoptosis	[210]
MetaCell	Fe^{3+}; Cu^{2+}; 2-Aminoterephthalic acid, thermosensitive liposome	Breast cancer	4T1 tumor	PTT		MetaCell effectively evades the immune system, penetrates tumors, and maintains stability under various conditions. MetaCell instigates cuproptosis and ferroptosis, resulting in substantial efficacy against cancer cells in vitro and in vivo	[211]

Table 3 (continued)

Nanomedicine	Components	Cancer type	Materias (cell lines)	Type of dynamic therapy	Combined drugs	Function description	Refs.
ECPCP	Elesclomol–Cu, cinnamaldehyde, polyethylene glycol	Breast cancer	4T1 tumor			ECPCP significantly prolongs the systemic circulation of elesclomol–Cu, enhances tumor accumulation, and induces cuproptosis. Cu^{2+}-stimulated Fenton-like reactions and cinnamaldehyde-stimulated ROS production simultaneously break redox homeostasis, inducing ICD of tumor cells and achieving cuproptosis and immunotherapy	[212]
CLDCu	Cu^{2+}, disulfiram, LMWH-TOS, chitosan	Melanoma	B16F10 tumor		aPD-L1	CLDCu induces cuproptosis by releasing Cu^{2+} and disulfiram and activates the STING pathway by releasing chitosan, potentiating DC maturation and evoking innate and adaptive immunity. CLDCu combined with aPD-L1 provokes stronger antitumor immunity	[213]
DCM@GDY-CuMOF@DOX	graphdiyne, CuMOF, DOX, DU145 cell membrane	Prostate cancer	DU145 cell tumor		DOX	DCM@GDY-CuMOF@DOX exhibits remarkable cell-killing efficiency by generating lethal ROS and mediating cuproptosis, effectively suppressing tumor growth in vivo without causing apparent side effects	[214]
O_2-PFH@CHPI NPs	Cu^{2+}; indocyanine green; O_2-saturated perfluorohexane	HCC	Huh7 tumor	PTT		Upon NIR, O_2-PFH@CHPI NPs accelerate catalytic reactions, trigger O_2 release for PDT, promote oxidative stress, and effectively activate through Cu^+-mediated cuproptosis. The redox balance tilt promotes lipid peroxidation and GPX4 inactivation, resulting in augmented ferroptosis	[215]
PCB	Cu-doped polypyrrole nanoparticles BPTES, platelet membrane	Breast cancer	4T1 tumor			PCB amplifies oxidative stress and induces DLAT oligomerization by releasing CuP, resulting in cuproptosis, which is enhanced by GLS1 inhibitor BPTES. PCB induces ICD, promoting immune cell infiltration into the tumor	[216]

Table 3 (continued)

GOx glucose oxidase, *PTT* photothermal therapy, *PDT* photodynamic therapy, *CDT* chemodynamic therapy, *HMSNs* hollow mesoporous silica nanoparticles; *ATP7A/B* ATPase copper transporting α/β, *CRC* colorectal cancer, *DTPH* 3,3'-dithiobis (propionohydrazide), *BSA* bovine serum albumin, *NaDTC* sodium diethyldithiocarbamate trihydrate, *BSO* butythione sulfoxideimine, *DDM* dodecyl-beta-D-maltoside, *ROS* reactive oxygen species, *GBM* glioblastoma, *TME* microenvironment, *PD-L1* programmed death-ligand 1, *aPD-L1* anti- PD-L1 antibody, *AMPK* adenosine 5'-monophosphate (AMP)-activated protein kinase, *CuET* copper (II) bis (diethyldithiocarbamate), *TIME* tumor immunosuppressive microenvironment, *ICD* immunogenic cell death, *DBCO* dibenzo-[g,p]chrysene-2,3,6,7,10,11,14,15-octaol, *CHO* cholesterol oxidase, *PSDA* 2,2'-(propane-2,2-diylbis(thio))diacetic acid linker, *OPDEA* 2-(N-oxide-N,N-diethylamino)ethyl methacrylate, *TPM* TP0751 peptide appended stem cell membrane, *SDT* sonodynamic therapy, *HSCLC* human small-cell lung carcinoma, *HCC* hepatocellular carcinoma, *NAC* N-acetyl-l-cysteine, *ECM* extracellular matrix, *DCs* dendritic cells, *TAMs* tumor-associated macrophages, *TCA* tricarboxylic acid, *HMnO₂* hollow manganese dioxide nanoparticles, *PDAC* pancreatic ductal adenocarcinoma, *MSN* dendritic mesoporous silica nanoparticles, *DMONs* dendritic mesoporous organosilica nanoparticles, *HPB* hollow mesoporous Prussian blue nanoparticles, *HCN* heterogeneous carbon nitride, *CuMS* copper loaded metallic molybdenum bisulfide nanosheets, *BETA* bis(2-((2-(ethylthio)ethyl)thio)ethyl)amine, *HBO* hyperbaric oxygen, *HIF-1* hypoxia-inducible factor 1, *NIR* near-infrared, *DOX* doxorubicin, *5-FU* 5-fluorouracil, *CuMOF* copper-based metal–organic framework, *LMWH-TOS* low molecular weight heparin-tocopherol succinate, *ZnPc* zinc phthalocyanine, *1-MT* 1-methyl tryptophan, *ITM* immunosuppressive tumor microenvironment, *GPX4* glutathione Peroxidase 4

while delivering Cu^{2+} to tumor tissues, thus inducing apoptosis and cuproptosis in tumor cells [207].

In addition, unlike drug treatment of vitro cultured cells, nanomedicine delivered to tumor tissues impacts the tumor microenvironment (TME), an important factor influencing tumor therapy outcomes, especially in tumor immunotherapy. Nanomedicine containing Cu relies on the TME for precise delivery to tumor tissues and has the potential to modify the TME, making it more conducive to tumor therapy (Table 3). For instance, PDA-DTC/Cu NPs trigger cuproptosis in tumor cells and facilitate the repolarization of tumor-associated macrophages to mitigate the tumor immunosuppressive microenvironment (TIME) [188]. Similarly, ES@CuO promoted cuproptosis-driven immune responses and remodeled the TIME by enhancing lymphocyte infiltration and increasing the release of inflammatory cytokines within tumors. The synergistic application of ES@CuO with programmed cell death-1 (PD-1) immunotherapy markedly enhanced anti-tumor efficacy in murine melanoma models [196]. Besides, CQG NPs induce cuproptosis and pyroptosis by disrupting antioxidant defense mechanisms within tumor cells [201]. This dual action facilitates the transformation of the TIME, augments the infiltration of immune cells into the tumor, and triggers a robust systemic immune response.

Furthermore, the integration of nanomedicine with dynamic therapies, such as photothermal therapy (PTT), photodynamic therapy (PDT), and chemodynamic therapy (CDT) significantly enhances the precision and efficacy of tumor treatments. This approach represents a promising research direction for future studies on cuproptosis-associated therapeutic strategies (Table 3). For instance, PTT can augment *E. coli*@Cu_2O-induced ferroptosis and cuproptosis, reversing the immunosuppression of colon tumors by initiating dendritic cell maturation and T-cell activation [205]. Through PTT and CDT, PEG@Cu2O-ES can generate ROS to target the ATP-Cu pump, reducing the efflux of Cu ions and exacerbating cuproptosis [208]. In nanomedicine, multiple antitumor components can be incorporated, significantly enhancing the medication's plasticity and multifunctionality. For instance, the CCNAs constructed by Wen et al., in addition to Cu^{2+}, contain zinc phthalocyanine (ZnPc), 1-methyl tryptophan (1-MT), and doxorubicin (DOX) [209]. Upon near-infrared laser irradiation, ZnPc released into tumor tissues exhibited a photodynamic effect that generated ROS, effectively promoting the release of DOX and inducing apoptosis while intensifying cuproptosis [209]. Moreover, the release of 1-MT from CCNAs can reverse TIME by inhibiting IDO-1-mediated Trp degradation, triggering an immunogenic cell death (ICD) response [209].

Although nanomedicine offers broad prospects for exploring tumor treatments, numerous issues must be addressed before clinical application, warranting attention in future studies on tumor treatments based on cuproptosis. First, current experimental studies are primarily conducted in animal tumor models, which differ from primary or metastatic tumors in humans, especially regarding the tumor microenvironment. Second, the efficacy of laboratory nanomedicine in delivering human tumor tissues remains unknown. Third, research on cuproptosis-related nanomedicine has mainly focused on a limited array of cancer types in animal models, such as breast and colorectal cancers. Expanding studies on other types of tumors, particularly those that are difficult to treat, is necessary to broaden the scope of targeting cuproptosis for tumor treatment. Lastly, biosafety is a critical concern, representing a significant factor in the transition of experimental drugs to clinical applications.

Sensitization of tumor cell cuproptosis
Targeting cell metabolism sensitizes tumor cell cuproptosis
Given the close relationship between cuproptosis and cellular metabolism, targeting cellular metabolism presents a strategy to sensitize tumor cells to cuproptosis. Cuproptosis is strongly associated with mitochondrial metabolism [9]. Elevated mitochondrial metabolism can sensitize tumor cells to Cu-induced cell death. Inhibition of the mitochondrial ETC or pyruvate uptake diminishes tumor cell responsiveness to Cu ionophores [9]. This suggests that inducing cuproptosis could effectively inhibit tumor growth in cells with high aerobic respiration levels, such as melanoma [217] and leukemia [218]. Additionally, a high mitochondrial metabolic state is characteristic of tumor cell resistance to certain drugs such as proteasome inhibitors [9, 219], cisplatin [220], and 5-FU [221]. Thus, tumor cells that exhibit resistance to these drugs may be more sensitive to Cu ionophores, and inducing cuproptosis may improve treatment outcomes in patients with acquired drug resistance.

Furthermore, high glycolysis levels are considered unfavorable for cuproptosis [9], indicating that targeting glycolysis in tumor cells could also be a method to induce or sensitize cuproptosis. For instance, 4-OI attenuates aerobic glycolysis in CRC cells by targeting GAPDH, thereby sensitizing them to cuproptosis induced by elesclomol–Cu [161]. Aerobic glycolysis is the primary energy source for most tumor cells, and inhibiting glycolysis can suppress their growth [222]. This suggests that inducing cuproptosis may enhance the inhibitory effect on tumor growth during aerobic glycolysis-targeted tumor therapy. Besides, recent studies have linked cuproptosis to protein synthesis, although the mechanism remains unclear. Liu et al. found that in elesclomol–Cu-treated cancer cells,

PPP1R15A could promote protein synthesis by downregulating eIF2α phosphorylation and upregulating 4EBP1 phosphorylation, thereby exacerbating proteotoxic stress [223], suggesting tumor cells with the high rate of protein synthesis might be more sensitive to cuproptosis inducers. Thus, although the relationship between cuproptosis and cell metabolism remains relatively vague and requires more basic and clinical research, the differential response of cuproptosis under various cellular metabolic states will help develop effective clinical application strategies.

Targeting cuproptosis regulatory proteins and pathways sensitizes tumor cell cuproptosis

Tsvetkov et al. identified several genes regulating cuproptosis. Among the proteins encoded by these genes, FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, and PDHB positively regulated cuproptosis, whereas MTF1, GLS, and CDKN2A negatively regulated it [9]. This suggests that these gene expression levels could serve as biomarkers of the sensitivity of tumor cells to cuproptosis. For instance, since MTF1, GLS, and CDKN2A knockout promoted cuproptosis in tumor cells, targeting these proteins or related signal pathways could represent a therapeutic sensitization strategy. Fan et al. found that plicamycin can inhibit head and neck squamous cell carcinoma (HNSCC) cell growth by targeting CDKN2A, implying that plicamycin may be a potential sensitizing agent for cuproptosis [224].

The occurrence of cuproptosis is dependent on Cu over-accumulation within cells. As previously mentioned, various factors, such as SLC31A1 and ATP7A/B, regulate cellular Cu levels [225, 226]. Since ATP7A/B can release Cu into the extracellular environment via the vesicle system [225], targeting ATP7A/B may be a means of inducing or sensitizing tumor cells to cuproptosis. For instance, Tsvetkov et al. found that in a Wilson disease mouse model, deletion of ATP7B resulted in excess Cu accumulation and cuproptosis in aging livers [9]. Additionally, Zhang et al. used nanoparticles to simultaneously deliver Cu and ATP7A-specific siRNA to small-cell lung cancer brain metastasis tumors, enhancing cuproptosis and tumor growth suppression [178]. Although an increase in intracellular Cu can promote ATP7A/B-dependent Cu efflux, Cu ionophores have been found to reduce ATP7A/B expression levels. For example, elesclomol and disulfiram could reduce the ATP7A expression level in CRC and prostate cancer cells, respectively [55, 139]. This suggests that Cu ionophores can transport Cu into cells while reducing Cu efflux, providing favorable conditions for inducing cuproptosis.

Although research into the mechanisms of cuproptosis remains in its initial stages, recent studies have uncovered several key regulatory factors of cuproptosis.

For instance, in hepatocellular carcinoma (HCC) cells, maternal embryonic leucine zipper kinase (MELK) can enhance DLAT expression by activating the PI3K/mTOR signaling pathway, thereby augmenting mitochondrial function [227]. MELK overexpression exacerbates elesclomol–induced cuproptosis and enhances its anti-tumor effects [227]. Mucin 20 (MUC20) overexpression in proteasome inhibitor-resistant multiple myeloma cells can induce cuproptosis, which is associated with the reduced cuproptosis inhibitor CDKN2A expression level [228]. Elesclomol–Cu activates the adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) signaling pathway in non-small cell lung cancer (NSCLC) cells, attenuating the cuproptosis [229]. Knocking down or inhibiting AMPK can aggravate elesclomol–Cu-induced cuproptosis [229]. Besides, in gastric cancer cells, METTL16 can promote cuproptosis by facilitating FDX1 accumulation via m6A modification of *FDX1* mRNA [230]. Further studies revealed that Sirtuin 2 (SIRT2) can inhibit METTL16 activity via deacetylation, and inhibiting SIRT2 can significantly enhance the anti-tumor effect of elesclomol–Cu in gastric cancer [230]. Additionally, some cuproptosis inhibitory factors have been identified in different tumor cells, such as SLC7A11 [231] and AT-rich interactive domain 1A (ARID1A) [232] in liver cancer cells, trigger transposable element-derived 1 (TIGD1) in CRC [233], and Wiskott-Aldrich syndrome protein family member 2 (WASF2) in ovarian cancer cells [234]. Targeting these factors may sensitize tumor cell cuproptosis and enhance the efficacy of tumor treatment.

Recently, extracellular signaling molecules have also been found to be involved in regulating cuproptosis. For example, in diabetic mice, an increase in blood advanced glycosylation end products (AGEs) and Cu upregulates SLC31A1 expression level in cardiomyocytes, thereby disturbing Cu homeostasis and promoting cuproptosis [235], suggesting that AGEs may be sensitizers for cuproptosis. Moreover, adrenomedullin (ADM), a member of the amylin/calcitonin gene-related peptide superfamily, has been found to promote the phosphorylation and nuclear translocation of Forkhead box O3 (FOXO3) via the p38/MAPK signaling pathway, thereby inhibiting FDX1 transcription and suppressing cuproptosis in renal cell carcinoma (RCC), promoting chemoresistance [236]. Consequently, targeting cuproptosis-related cytokines in the blood is also a strategy to sensitize tumor cells to cuproptosis. Table 4 lists the regulatory proteins and pathways of cuproptosis, which are potential targets for sensitizing Cu-induced tumor cell death.

Targeting cuproptosis to overcome tumor drug resistance
As the duration of drug usage extends, tumor cells may develop resistance to therapeutic agents, diminishing

Table 4 Cuproptosis regulatory proteins and pathways

Proteins/pathways	Effect on cuproptosis	Potential inhibitor/ agonist	Cancer type	Materials (cell lines)	Role in cuproptosis and its involved mechanism	Refs.
MTF1	Negative		Ovarian cancer	OVISE	Knockout of MTF1 enhances elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
GLS	Negative		Ovarian cancer	OVISE	Knockout of GLS enhances elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
		BPTES	Breast cancer	4T1 tumor	BPTES can enhancing PBC, a nanomedicine containing Cu and BPTES, -induced cuproptosis by inhibiting GLS1 activity	[216]
CDKN2A	Negative		Ovarian cancer	OVISE	Knockout of CDKN2A enhance elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
		Plicamycin	HNSCC	TU212	Plicamycin can inhibit HNSCC cell growth by targeting CDKN2A	[224]
FDX1	Positive		Ovarian cancer, lung cancer	OVISE, ABC1	FDX1 reduce Cu^{2+} to Cu^+. FDX1 is an upstream regulator of protein lipoylation. Knockout of FDX1 suppresses elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
LIAS	Positive		Ovarian cancer, lung cancer	OVISE, ABC1	Knockout of LIAS suppresses elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
LIPT1	Positive		Ovarian cancer	OVISE	Knockout of LIPT1 suppresses elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
DLD	Positive		Ovarian cancer	OVISE	Knockout of DLD suppresses elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
DLAT	Positive		Ovarian cancer	OVISE	Cu directly binds and promotes the oligomerization of lipoylated DLAT, resulting in proteotoxic stress. Knockout of DLAT suppresses elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
PDHB	Positive		Ovarian cancer	OVISE	Knockout of PDHB suppresses elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
PDHA1	Positive		Ovarian cancer	OVISE	Knockout of PDHA1 suppresses elesclomol–Cu and disulfiram–Cu-induced cell death	[9]
SLC31A1	Positive				Overactivaiton of SLC31A1 enhances intracellular Cu accumulation	[225, 226]
ATP7A/B	Negative				Knockout of ATP7A/B enhances intracellular Cu accumulation	[225, 226]

Table 4 (continued)

Proteins/pathways	Effect on cuproptosis	Potential inhibitor/ agonist	Cancer type	Materials (cell lines)	Role in cuproptosis and its involved mechanism	Refs.
GAPDH	Negative	4O-I	CRC	HCT116, LoVo	4-OI enhances elesclomol–Cu-mediated cuproptosis by targeting GAPDH to suppress glycolysis	[161]
PPP1R15A	Positive		Breast cancer, prostate cancer, lung cancer	BT-549, PC-3, DU145, A549	Elesclomol–Cu treatment upregulates PPP1R15A, promoting proteotoxic stress by enhancing EIF2S1 and 4E-BP1-associated translation initiation	[223]
MELK	Positive		HCC	Huh7	MELK augments the expression of DLAT through the PI3K/mTOR signaling pathway and promotes mitochondrial function, which subsequently promotes the progression of HCC. MELK overexpression significantly enhances the anti-tumor effect of elesclomol in HCC	[227]
MUC20	Positive		MM	KAS-6/1/U266	MUC20 attenuated proteasome inhibitor resistance in MM cells by inducing cuproptosis via the inhibition of CDKN2A expression	[228]
AMPK	Positive	Dorsomorphin	NSCLC	Calu1 cells	Elesclomol–Cu activates AMPK. Knockdown or inhibition of AMPK suppresses elesclomol–Cu-induced cell death	[229]
METTL16	Positive		Gastric cancer	HGC-27 cell	METTL16 promotes cuproptosis by facilitating FDX1 accumulation via m6A modification on *FDX1* mRNA	[230]
SIRT2	Negative	AGK2	Gastric cancer	HGC-27 cell	SIRT2 delactylates METTL16-K229 to inhibit the METTL16 activity. Combining elesclomol with SIRT2-specific inhibitor AGK2 enhance cuproptosis in gastric tumors in vitro and in vivo	[230]
SLC7A11	Negative	SASP/erastin	HCC	Huh7/SMMC-7721	Disulfiram–Cu induces ferroptosis and cuproptosis while compensatorily activating cellular SLC7A11 expression by inhibiting ubiquitination-proteasome degradation. Inhibition or knockdown of SLC7A11 promotes disulfiram–Cu-induced ferroptosis and cuproptosis in HCC cells	[231]

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Table 4 (continued)

Proteins/pathways	Effect on cuproptosis	Potential inhibitor/ agonist	Cancer type	Materials (cell lines)	Role in cuproptosis and its involved mechanism	Refs.
ARID1A	Negative		HCC	Hep3B, HepG2, PDX(ARID1A-wt/mutant)	ARID1A loss shifts cellular glucose metabolism from aerobic glycolysis to dependence on the TCA cycle and oxidative phosphorylation. ARID1A-deficient HCC cells and xenograft tumors are highly sensitive to Cu treatment	[232]
TIGD1	Negative		CRC	HCT116	TIGD1 knockdown can promote cuproptosis in HCT116 cells	[233]
WASF2	Negative		Ovarian cancer	A2780; TOV-21G; OVCA429	WASF2 knockdown significantly enhances the elesclomol–Cu-induced cell death	[234]
G6PD	Negative		ovarian endometriomas	EESCs, 11Z	Elesclomol–Cu treatment induces the interaction of FDX1 and G6PD, promoting G6PD degradation and GSH reduction, thereby intensifying cuproptosis	[237]
Adrenomedullin	Negative		ccRCC	A498, 786-0	Adrenomedullin promotes the phosphorylation and nuclear translocation of FOXO3 through the p38/MAPK signaling pathway, thereby inhibiting the transcription of FDX1 and suppressing cuproptosis in RCC cells	[236]
p32	Positive		ccRCC	ACHN, 786-O	p32 promotes lipo-DLAT oligomerization by directly binding Cu. Combinational treatment of p32 and elesclomol–Cu inhibits ccRCC progression through disruption of TCA cycle	[238]

MTF1 metal-regulatory transcription factor-1, *GLS* glutaminase, *CDKN2A* cyclin-dependent kinase inhibitor 2A, *FDX1* ferredoxin 1, *DLAT* dihydrolipoamide S-acetyltransferase, *LIAS* LA synthase, *LIPT1* lipoyl transferase 1, *DLD* dihydrolipoamide dehydrogenase, *PDHA1* pyruvate dehydrogenase E1 subunit alpha 1, *PDHB* pyruvate dehydrogenase E1 subunit beta, *HNSCC* head and neck squamous cell carcinoma, *SLC31A1, SLC31A1* the solute carrier family 31 member 1, *ATP7A/B* ATPase copper transporting α/β, *SLC7A11* solute carrier family 7 membrane 11, *MUC20* Mucin 20, *MM* multiple myeloma, *MELK* maternal embryonic leucine zipper kinase, *HCC* hepatocellular carcinoma, *ARID1A* AT-rich interactive domain 1A, *TCA* tricarboxylic acid, *SIRT2* Sirtuin 2, *AMPK* adenosine 5′-monophosphate (AMP)-activated protein kinase, *NSCLC* non-small cell lung cancer, *TIGD1* trigger transposable element-derived 1, *CRC* colorectal cancer, *WASF2* Wiskott-Aldrich syndrome protein family member 2, *EESCs* ectopic endometrial stromal cells, *G6PD* glucose-6-phosphate dehydrogenease, *FOXO3* Forkhead box O3, *MAPK* mitogen-activated protein kinase, *ccRCC* clear cell renal cell carcinoma

drug efficacy and leading to tumor relapse or progression. Therefore, effectively overcoming cancer drug resistance has always been a significant theme in cancer treatment research. Since cuproptosis has been established as a novel mode of cell death, targeting tumor cell cuproptosis holds potential as a new strategy to overcome tumor drug resistance.

The use of Cu ionophores to overcome tumor chemotherapeutic drug resistance has a long history of research. On one hand, Cu ionophores can transport Cu into the cells to induce Cu-triggered cell damage, thereby exacerbating the death of resistant cells (Table 1). For instance, in prostate cancer, elesclomol–Cu can enhance sensitivity to docetaxel by inducing DLAT/mTOR pathway-dependent cuproptosis in vitro and in vivo [87]. Disulfiram–Cu has been found to sensitize breast cancer cells to paclitaxel by simultaneous induction of ROS and inhibition of NF-κB, suggesting its potential to overcome clinical resistance to paclitaxel [108]. Disulfiram–Cu can enhance the cytotoxicity of gemcitabine by reversing

NF-κB activity in gemcitabine-resistant colon cancer cells [111]. Moreover, disulfiram–Cu was found to overcome bortezomib and cytarabine resistance in cell lines from patients with Down syndrome-associated acute myeloid leukemia, which is thought to be associated with the induction of apoptosis and re-inhibition of proteasome activity [138]. In addition to Cu ionophores, small molecule compounds capable of inducing cuproptosis are potential candidates for overcoming tumor treatment resistance. These compounds usually enhance anti-tumor effects by inducing cell damage through multiple mechanisms. For example, zinc pyrithione induces necrosis in prostate cancer cells by activating the PKC and ERK pathways and enhancing ROS production [239]; 4-OI induces ferritinophagy-dependent ferroptosis in multi-drug resistant retinoblastoma cells [240]. Among these compounds, the ferroptosis inducer erastin has garnered significant attention for its ability to reverse the resistance of various tumor cells to chemotherapeutic drugs, including ovarian cancer [241, 242], AML cells [243], NSCLC cells [244], and prostate cancer cells [245]. However, whether cuproptosis contributes to the sensitizing effects of these drugs requires further investigation. Future research should explore combining these compounds with chemotherapeutic drugs to treat drug-resistant tumor cells that are sensitive to cuproptosis. Additionally, nanomedicine-based targeting of cuproptosis can be examined to overcome chemotherapeutic drug resistance. For instance, CuO_2/DDP@SiO_2, which releases Cu^{2+} and cisplatin, can induce cuproptosis and block the entire cisplatin efflux pathway by downregulating multidrug resistance-associated protein 2 (MRP2), enhancing the anti-tumor effect of cisplatin [193]. E-C@DOX NPs can inhibit tumor cell stemness and cell survival-related pathways while working with Cu ions to damage mitochondria and induce cuproptosis, suppressing the ATP-dependent drug efflux pathway and reversing DOX resistance [204].

Notably, Cu ionophores also chelate certain drugs, especially platinum-based drugs, which are widely used as first-line clinical treatments for cancer. Disulfiram has been found to form a new platinum (Pt) chelate, $Pt(DDTC)^{3+}$, which has a stronger anti-NSCLC effect than cisplatin alone [105]. Furthermore, Pt drugs and Cu share molecular mechanisms for intracellular transport and extracellular efflux, such as the Cu transporter CTR1, which can transport Pt into the cell [246, 247], and the Cu chaperone protein ATOX1, which can deliver Pt to ATP7A/B located on TGN, thereby promoting the efflux of Pt and leading to drug resistance during treatment [248–250]. Thus, targeting Cu homeostasis can also alter the intracellular Pt drug concentration, a potential mechanism for overcoming resistance. Yuki et al. found that in bladder cancer cells treated with disulfiram and cisplatin, disulfiram could reduce ATP7A expression level and its localization in the TGN, accumulating intracellular cisplatin and enhancing tumor cell death [251]. However, increased Cu levels reduce the expression or activity of Cu uptake proteins, such as CTR1, and enhance the translocation of ATP7A/B from the Golgi to post-Golgi sites or lysosomes to promote Cu efflux, which may be unfavorable for accumulating Pt drugs within cells [252–255]. Therefore, targeting Cu homeostasis to overcome tumor cell resistance to Pt drugs requires comprehensive consideration of the dosage of Pt drugs and the action of cuproptosis.

Moreover, targeted cuproptosis could be used to solve the problem of drug resistance in targeted therapies. For example, disulfiram–Cu kills and sensitizes BRAF-mutant thyroid cancer to BRAF kinase inhibitor by relieving feedback activation of the MAPK/ERK and PI3K/AKT pathways in a ROS-dependent manner [134]. In HCC cells, researchers observed that disulfiram–Cu could strengthen the cytotoxicity of sorafenib by simultaneously inhibiting the NRF2 and MAPK kinase signaling pathways and arrest tumor growth in vitro and in vivo [136]. Current research on cuproptosis overcoming drug resistance in tumor-targeted therapy remains relatively limited. One of the main reasons is that the molecular regulation mechanism underlying cuproptosis remains unknown. However, the relationship between tumor drug resistance and cuproptosis remains unclear, hindering the progress of targeting cuproptosis to overcome drug resistance in tumor-targeted therapy.

Targeting cuproptosis to enhance tumor immunotherapy

Immunotherapy has become an important clinical strategy for cancer treatment due to its significant efficacy in tumor therapy. Since Cu metabolism and cuproptosis play crucial regulatory roles in tumor immunity, targeting cuproptosis may represent a vital sensitization strategy for tumor immunotherapy. PD-1/PD-L1 (Programmed death-ligand 1) serves as a crucial immune checkpoint, and inhibiting or eliminating PD-1/PD-L1 can lead to favorable clinical outcomes in patients with cancer [256]. Cu may exert a positive regulatory effect on PD-L1 expression in tumors. For instance, disulfiram–Cu can upregulate PD-L1 expression in HCC cells by inhibiting Poly (ADP-ribose) polymerase 1 (PARP1) activity and promoting glycogen synthase kinase 3β (GSK-3β) phosphorylation, thereby suppressing T-cell infiltration [135]. Hence, simultaneous targeting of cuproptosis and co-administration of PD-1/PD-L1 inhibitors may enhance therapeutic effects. This notion is supported by preclinical studies demonstrating superior tumor growth inhibition when

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Cu ionophores were combined with Cu and anti-PD-L1 agents in PDAC [102], lung cancer [106], and HCC cells [135].

The TME is a pivotal determinant of the efficacy of tumor immunotherapy. Reshaping the immunosuppressive TME, such as dendritic cell maturation and activation of CD8^{+} T cells, is poised to enhance tumor suppression [257]. The cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling pathway is a critical component of innate immunity, capable of sensing aberrant DNA and triggering the release of type I interferons, thereby promoting dendritic cell maturation and migration, as well as augmenting the cytotoxic effects of T lymphocytes or natural killer cells [258]. Jiang et al. demonstrated that elesclomol–Cu-induced cuproptosis in clear cell RCC can activate the cGAS-STING pathway within dendritic cells, thereby promoting the release of inflammatory mediators, including IFN-γ, TNF-α, IL2, C-X-C motif chemokine ligand 10 (CXCL10), and CXCL11, ultimately enhancing tumor immunotherapy [259]. Additionally, CS/MTO-Cu@AMI, established by Huang et al., can activate anti-tumor immunity by inducing dsDNA damage and activating the cGAS-STING pathway [173]. Similarly, PCM nanoinducers constructed by Dai et al. can trigger the release of mitochondrial DNA during inducing tumor cell cuproptosis, activating the cGAS-STING pathway and stimulating innate and adaptive immune responses, thereby enhancing tumor suppression [184].

As discussed in this review, nanomedicine-based cuproptosis induction systems mediate tumor cell cuproptosis and impact the TME by depleting GSH, aggravating oxidative stress, and inducing other types of cell damage, such as pyroptosis and apoptosis. These alterations result in the remodeling of the TME and initiation of ICD responses, which are conducive to enhanced immunotherapy. Consequently, inducing cuproptosis may represent an effective strategy for sensitizing tumors to immunotherapy. Table 3 summarizes various nanomedicines, such as NP@ESCu [171], BCMD [176], OMP [186], PCD@CM [195], ES@CuO [196], CBS [203], *E. coli*@Cu_2O [205], PEG@Cu_2O-ES [208], and CLDCu [213], in combination with anti-PD1 or anti-PD-L1 antibodies, effectively inhibited tumor growth. However, several issues must be addressed before these strategies are applied clinically. For instance, the mechanisms underlying cuproptosis-mediated TME remodeling remain unclear. Additionally, are the induction conditions and regulatory mechanisms of cuproptosis in tumors and immune cells similar or different? How can we target tumor cell cuproptosis more precisely to initiate ICD responses? Addressing these problems holds promise for improving the effectiveness of tumor immunotherapy.

Conclusions and future perspectives

Cuproptosis, characterized by its unique features, represents a novel mode of cell death that has infused new optimism into cancer treatment. Since its conceptualization, cuproptosis has received significant attention in oncology. Conversely, it has emerged as a promising therapeutic target, with ongoing research poised to unveil additional cuproptosis inducers, including small-molecule compounds and nanomedicines. This expanding repertoire of therapeutic options holds the potential to diversify tumor treatment strategies. Targeting cuproptosis offers a novel approach to combatting tumor drug resistance. Exploiting therapeutic-induced metabolic changes in tumor cells, such as heightened mitochondrial metabolism and glycolysis, rendering them susceptible to cuproptosis, provides a pathway to sensitize tumor cells to drug interventions or overcome drug resistance. Furthermore, inducing cuproptosis in tumor tissues can remodel the tumor microenvironment, fostering dendritic cell maturation and immune cell infiltration. Consequently, targeting cuproptosis promises to enhance the response rates and overcome resistance to immunotherapy.

However, understanding and research regarding cuproptosis are still in their infancy. Before practical application, numerous issues must be addressed. For instance, Cu can play a dual role in tumor initiation and progression, promoting tumorigenesis and inducing cell death [260, 261]. Cu chelators reduce Cu bioavailability and exert anticancer effects [262]. Therefore, comprehending how tumor cells balance the dual effects of Cu is crucial. Furthermore, whether cuproptosis or its related signaling pathways have pro-tumor effects during tumor initiation, development, and treatment remains unclear. In addition, reliable biomarkers, initiation mechanisms, and links with other cell death forms for cuproptosis remain lacking, impeding the progress of cuproptosis-associated research in diseases and targeted clinical applications. Moreover, distinguishing between the regulatory mechanisms of cuproptosis in normal and tumor cells is crucial for improving the precision of cuproptosis-targeted therapy and reducing the side effects of treatment. Additionally, currently widely used cuproptosis inducers, such as Cu ionophores elesclomol and disulfiram, have not revealed promising therapeutic effects in clinical trials.

Based on the challenges outlined above, the following recommendations may help promote the translation of cuproptosis-associated anti-tumor therapies from the laboratory to clinical practice in future research. First, unraveling the molecular mechanisms underlying tumor cell tolerance and exploiting high Cu levels may offer insights into inducing cuproptosis by disrupting

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endogenous Cu metabolism within tumor cells. For example, hepatocytes are the primary storage cells for Cu, and abnormal Cu accumulation in liver cells, as observed in patients with Wilson's disease, promotes the development of HCC [263]. Accordingly, driving accumulated Cu in HCC cells to induce cuproptosis might reverse the pro-carcinogenic effects of Cu to anticarcinogenic effects. Additionally, future research and development efforts should focus on novel cuproptosis inducers or induction strategies, including natural products, small-molecule compounds, and nanomedicine. Furthermore, improving the precision of drug delivery to tumor cells and the stability of drugs in plasma should be a priority in the development of cuproptosis inducers. Moreover, conducting more clinical trials is crucial for promoting the clinical application of cuproptosis-targeted therapies. For example, combining Cu ionophores with frontline clinical drugs that can enhance sensitivity to cuproptosis might address issues of drug resistance during treatment. Finally, similar to other types of cell death, inducing cuproptosis to treat tumors will also face the drug resistance issues. Therefore, uncovering the mechanisms of cuproptosis-related drug resistance, particularly in different tumor cell types, should be a key focus of future research.

In summary, cuproptosis is a new target in cancer treatment. As the regulatory mechanisms of cuproptosis continue to be elucidated and the efficiency of cuproptosis induction methods improves, targeting cuproptosis presents a promising new approach to combat chemotherapy and immunotherapy resistance in cancer treatment, leading to improved therapeutic outcomes.

Abbreviations

ROS	Reactive oxygen species
DLAT	Dihydrolipoamide S-acetyltransferase
TCA	Tricarboxylic acid
STEAP	Six-transmembrane epithelial antigen of the prostate
DCYTB	Duodenal cytochrome b
CTR1	Cu transport protein 1
SLC31A1	The solute carrier family 31 member 1
ATP7A	ATPase copper transporting alpha
MT1/2	Metallothionein1/2
GSH	Glutathione
SOD1	Superoxide dismutase 1
ATOX1	Antioxidant 1
TGN	Trans-Golgi network
COX	Cytochrome oxidase
OXPHOS	Oxidative phosphorylation
MEK	Mitogen-activated extracellular signal-regulated kinase
MAPK	Mitogen-activated protein kinase
FGFs	Fibroblast growth factors
VEGF	Vascular endothelial growth factor
TNF-α	Tumor necrosis factor alpha
NAC	*N*-Acetylcysteine
TNBC	Triple negative breast cancer
AMPK	Adenosine 5′-monophosphate (AMP)-activated protein kinase
ES	Elesclomol
FDX1	Ferredoxin 1
ETC	Electron transport chain
LA	Lipoic acid
LIAS	LA synthase
LIPT1	Lipoyl transferase 1
DLD	Dihydrolipoamide dehydrogenase
PDHA1	Pyruvate dehydrogenase E1 subunit alpha 1
PDHB	Pyruvate dehydrogenase E1 subunit beta
MTF1	Metal-regulatory transcription factor-1
GLS	Glutaminase
CDKN2A	Cyclin-dependent kinase inhibitor 2A
RCD	Regulated cell death
CRC	Colorectal cancer
SLC7A11	Solute carrier family 7 membrane 11
PDAC	Pancreatic ductal adenocarcinoma
ER stress	Endoplasmic reticulum stress
DSF	Disulfiram
ULK	Unc-51-like autophagy activating kinase
5-FU	5-Fluorouracil
4-OI	4-Octyl itaconate
HCC	Hepatocellular carcinoma
TME	Microenvironment
TIME	Tumor immunosuppressive microenvironment
PD-1	Programmed cell death-1
PD-L1	Programmed death-ligand 1
PTT	Photothermal therapy
PDT	Photodynamic therapy
CDT	Chemodynamic therapy
DOX	Doxorubicin
ZnPc	Zinc phthalocyanine
1-MT	1-Methyl tryptophan
ICD	Immunogenic cell death
HNSCC	Head and neck squamous cell carcinoma
NSCLC	Non-small cell lung cancer
MELK	Maternal embryonic leucine zipper kinase
cGAS	Cyclic GMP-AMP synthase
STING	Stimulator of interferon genes
CXCL	C-X-C motif chemokine ligand

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C. Z. contributed to conception and design of the manuscript; C. Z. and T. H. drafted the manuscript and conducted image processing. L. L. contributed to reviewing. C. Z. given the final approval of the version to be published.

Availability of data and materials
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Declarations

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Not applicable.

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Competing interests
The authors declare no competing interests.

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