Cancer Cell International

REVIEW

Open Access

Cuproplasia and cuproptosis in hepatocellular carcinoma: mechanisms, relationship and potential role in tumor microenvironment and treatment

Ruoyu Zhang[1†], Yunfei Tan[3†], Ke Xu[1], Ning Huang[1], Jian Wang[4], Mei Liu[2*] and Liming Wang[1*]

Abstract

Background Hepatocellular carcinoma (HCC) is the main phenotype of liver cancer with a poor prognosis. Copper is vital in liver function, and HCC cells rely on it for growth and metastasis, leading to cuproplasia. Excessive copper can induce cell death, termed cuproptosis. Tumor microenvironment (TME) is pivotal in HCC, especially in immunotherapy, and copper is closely related to the TME pathogenesis. However, how these two mechanisms contribute to the TME is intriguing.

Main body We conducted the latest progress literature on cuproplasia and cuproptosis in HCC, and summarized their specific roles in TME and treatment strategies. The mechanisms of cuproplasia and cuproptosis and their relationship and role in TME have been deeply summarized. Cuproplasia fosters TME formation, angiogenesis, and metastasis, whereas cuproptosis may alleviate mitochondrial dysfunction and hypoxic conditions in the TME. Inhibiting cuproplasia and enhancing cuproptosis in HCC are essential for achieving therapeutic efficacy in HCC.

Conclusion An in-depth analysis of cuproplasia and cuproptosis mechanisms within the TME of HCC unveils their opposing nature and their impact on copper regulation. Grasping the equilibrium between these two factors is crucial for a deeper understanding of HCC mechanisms to shed light on novel directions in treating HCC.

Keywords Copper, Cuproplasia, Cuproptosis, Hepatocellular carcinoma, Mechanism, Tumor microenvironment

[†]Ruoyu Zhang and Yunfei Tan The co-first authors.

*Correspondence:
Mei Liu
liumei@cicams.ac.cn
Liming Wang
stewen_wang@sina.com
[1] Department of Hepatobiliary Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 17 Panjiayuan Nanli Area, Chaoyang District, Beijing 100021, China
[2] Laboratory of Cell and Molecular Biology & State Key Laboratory of Molecular Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, 17 Panjiayuan Nanli, Chaoyang District, P.O. Box 2258, 100021 Beijing, People's Republic of China
[3] State Key Laboratory of Holistic Integrative Management of Gastrointestinal Cancers, Beijing Key Laboratory of Carcinogenesis and Translational Research, Unit III, Gastrointestinal Cancer Center, Peking University Cancer Hospital & Institute, Beijing, China
[4] Department of Human Anatomy, School of Basic Medical Sciences, Zhengzhou University, Zhengzhou 450001, Henan, China

Introduction

Liver cancer poses a serious global health challenge and exhibits the most rapid rise in mortality in decades [1]. According to the latest cancer statistics in the United States, liver cancer ranks fourth in mortality among men and seventh among women [1]. Hepatocellular carcinoma (HCC) accounts for approximately 90% of cases, making it the predominant subtype of liver cancer [2]. Hepatitis B virus, hepatitis C virus infection, nonalcoholic steatohepatitis, aflatoxin exposure, liver cirrhosis, autoimmune liver disease, and metabolic syndrome are prominent risk factors for HCC, [3–6] especially Hepatitis B virus infection in China and nonalcoholic steatohepatitis in the West [7]. Despite advancements in drug development and treatment protocols, the prognosis of HCC patients has improved but remains relatively poor [8]. Furthermore, the specific molecular mechanisms underlying HCC are not well established, highlighting the pressing need for predictive biomarkers and novel targeted therapies for the diagnosis and treatment of HCC.

Copper is an essential trace element for the human body. Copper, serving as an indispensable coenzyme for metabolic enzymes, participates in a series of physiological processes such as respiration, lipolysis, cell growth, and proliferation [9]. Cancer, especially HCC, exhibits an increased demand for copper attributed to tumor growth and metastasis [9, 10]. Serum copper levels are notably higher in HCC patients compared to healthy individuals [11]. Elevated copper levels directly correlate with HCC progression, leading to cuproplasia [12]. Cuproplasia, characterized by copper-dependent cell growth and proliferation, manifests itself as copper-dependent tumor formation and development [9]. Nevertheless, cellular copper levels demonstrate a bidirectional, U-shaped dose–response relationship [13]. Copper's inherent redox properties render it beneficial yet potentially toxic to cells. Excessive extracellular and intracellular copper can lead to kidney disease, liver disease, and brain damage [14, 15]. Consequently, increased copper levels in HCC tissues also induce cuproptosis, potentially eliminating cancerous cells. Cuproptosis was first discovered by *Tsvetkov and his colleagues*, who identified a novel form of regulated cell death induced by copper, distinct from the known cell death modes such as necroptosis, apoptosis, and ferroptosis. They coined the term 'cuproptosis' [16]. This discovery has been published in *science* and has garnered considerable attention in scientific research. The prevalent hypoxic conditions within the tumor microenvironment (TME) influence copper metabolism in HCC tissue [17].

This review concentrates on elucidating the mechanisms of cuproplasia and cuproptosis in HCC, along with their implications in HCC prognosis, hoping this insight will steer novel avenues for the diagnosis and treatment of HCC.

Copper metabolism and role in HCC

Copper metabolism is primarily regulated by the liver [18]. Humans typically contain approximately 80 mg of copper, predominantly distributed among vital organs such as the liver, brain, and eyes [19]. Daily dietary intake provides 1.3 mg of copper, with 0.8 mg directed to hepatic circulation [19]. Copper is an indispensable trace metal, acting as a cofactor for various copper-dependent enzymes, notably respiratory enzymes in mitochondria (cytochrome c oxidase(COX)). Its involvement extends to crucial physiological processes such as lipolysis (phosphodiesterase 3B), [9] crosslinks of elastin and collagen (lysyl oxidase (LOX) and LOXL2), [20, 21] cell growth and proliferation (mitogen-activated protein kinase kinase 1 (MEK1) and MEK2), [9] autophagy (the kinases Unc-51 Like Autophagy Activating Kinase 1 (ULK1) and ULK2), [22] iron absorption and transport (ceruloplasmin), [23] signal transduction (dopamine β-hydroxylase), [24] reactive oxygen species (superoxide dismutase (SOD), glutathione (GSH)), epigenetic modification (LOXL2), leukocyte trafficking (amine oxidase copper containing 3), [25] as well as playing a role in cardiovascular, nervous, and immune systems [14]. Dynamic fluctuations in copper levels within the body orchestrate the changes in external stimuli and biological states to control and regulate biological functions, underscoring the significance of copper homeostasis in sustaining normal physiological processes.

However, copper ion homeostasis can be easily disrupted. Copper deficiency impairs the activity of copper-dependent enzymes, affecting energy metabolism, glucose tolerance, immune responses, and the antioxidant defense system, culminating in oxidative stress-induced damage. Conversely, excess copper can cause cell damage mainly by enhancing free radicals to exacerbate oxidative stress and DNA damage, ultimately fostering malignant transformations [26]. Notably, perturbations in copper homeostasis correlate with chemotherapy resistance and immune checkpoint dysregulation [27, 28]. Abnormal copper homeostasis may lead to neurodegenerative diseases [29], metabolic diseases [30], cardiovascular diseases [31], tumors [32] and other multi-system diseases. Studies have demonstrated the association between elevated serum copper levels and various tumors, [32] elucidating the multifaceted involvement of copper in tumorigenesis. For HCC fields, *Caroline I. Davis* et al. have demonstrated the vulnerability of copper homeostasis in HCC [33]. Increased serum copper content may promote the progression from cirrhosis to HCC [34]. *P. Dongiovanni* has shown that increased

copper concentration in HCC is positively correlated with oxidative stress, impacting the occurrence and development of tumors [35]. *C. Porcu*'s study has shown that high copper concentrations regulate the Copper Transport Protein 1 axis, promoting the growth, migration, and invasion of liver cancer cells [34]. *Xianglong Liu* et al. studied the differences between high copper and low copper phenotypes in HCC, showing that compared with low copper subtype, patients with high copper subtype had significantly abnormal immune function, a higher probability of gene mutation, and significantly weaker sensitivity and reactivity to chemotherapy drugs [36]. The specific mechanisms of copper in HCC are described as follows.

Cuproplasia and HCC

The mechanism of cuproplasia in HCC

Elevated copper levels pose a heightened risk of HCC, [34, 37] with HCC necessitating higher concentrations of copper than normal cells for tumor growth and proliferation [9]. Consequently, the concept of cuproplasia emerged. Cuproplasia is characterized by copper-dependent cell growth and proliferation, [9] encompassing neoplasia and hyperplasia, as well as the primary and secondary effects of copper [38]. The mechanism of cuproplasia has been described in Fig. 1. Mechanistically, existing studies have shown that cuproplasia drives tumor progression via the following mechanisms: (1) Elevated copper levels affect glycolysis, lipid metabolism, gluconeogenesis, collagen crosslinking, autophagy and other biological processes (2) Copper assists mitochondrial COX activity, promoting reactive oxygen species (ROS) production and tumor cell proliferation; [39, 40] (3) Copper regulation of signaling pathways like the Antioxidant protein 1 (ATOX)- Adenosine 5'-triphosphatase copper transporting alpha (ATP7A)-LOX pathway enhances metastasis and expansion; [41] (4) Copper activation of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF2), and tumor necrosis factor (TNF) promotes tumor angiogenesis; [9] (5) Copper-induced immune checkpoint expression, such as programmed death-ligand 1 (PD-L1), [42] aids in evading immune injury, ultimately leading to tumor development and chemotherapy resistance [36].

Copper is strongly implicated in the development of cirrhosis and HCC, contributing to neoplasia [10, 37, 43]. Patients with Wilson disease, an autosomal recessive disorder of copper metabolism, exhibit a significantly elevated incidence of HCC. Copper accumulation may promote malignant transformation and cell death of liver cells [33]. Prolonged exposure to high-soluble copper induces significant morphological changes in the liver, increased cell cycle arrest, and suppressed lymphocytes, [44] thereby elevating the frequency of gene mutation [43, 45]. Excessive copper exposure of liver cells could lead to the decrease of glucose 6-phosphate dehydrogenase (G6PDH) and GSH reductase, [46] excessive production of ROS, and decreased antioxidant function, which increases the probability of gene damage. Moreover, it may induce mitochondria-mediated liver cell death and apoptosis [18, 47]. Additionally, copper directly binds to dual-specificity protein kinases1/2 (DSPK1/2) with high affinity, and is closely related to the classical oncogenic pathways such as BRAF-RAS-RAF-DSPK1/2-extracellular-regulated kinase1(ERK1) and phosphatidylinositol 3-kinase (PI3K)-3-Phosphoinositide-dependent protein kinase1-protein kinase B (PKB) signaling pathways [48]. Consequently, the collective increase in the risk of genetic mutations contributes to neoplasia. In addition, studies have shown that blocking copper transporter-1(also known as copper importer solute carrier family 31 member 1 (SLC31A1)) or reducing intracellular copper levels could inhibit PI3K-PKB and mitogen-activated protein kinase (MAPK) signaling pathway, thereby suppressing neoplasia [49–51]. SLC31A1 represents an important potential therapeutic target in HCC.

Clinical research has demonstrated the increased tissue and intracellular copper accumulation in human HCC tissue samples, suggesting the necessity of copper for tumor proliferation [52]. Elevated serum copper levels correlate with poorer HCC survival [53]. Isotopic copper studies suggested that the increased copper burden in the TME is not sourced from dietary intake, but rather from the redistribution of copper to cysteine-rich proteins in the body [17]. Due to the substantial proliferation demands, HCC cells necessitate increased energy, supported by copper acting as a cofactor for various mitochondrial respiratory enzymes (e.g., COX, SOD1, ATOX1). Consequently, HCC cells require higher copper levels compared to normal cells for proliferation [9, 10, 43, 45]. Elevated copper levels enhance the function of the respiratory enzymes in mitochondria, thereby augmenting mitochondrial respiration.

Copper also promotes hyperplasia via non-mitochondrial pathways. For instance, copper degrades phosphodiesterase, altering the activity of 3',5'-cyclic AMP to stimulate lipolysis [54]. Dysregulated lipid metabolism is a hallmark of HCC cells [55]. The cAMP pathway facilitates the conversion of more triglycerides into glycerol and fatty acids, which can subsequently promote tumor proliferation. Additionally, copper alleviates the ULK1 and ULK2 pathways to enhance autophagic flux, [22] thus providing more copper-dependent targets for tumor proliferation by regulating protein quality. The copper metabolism MURR1

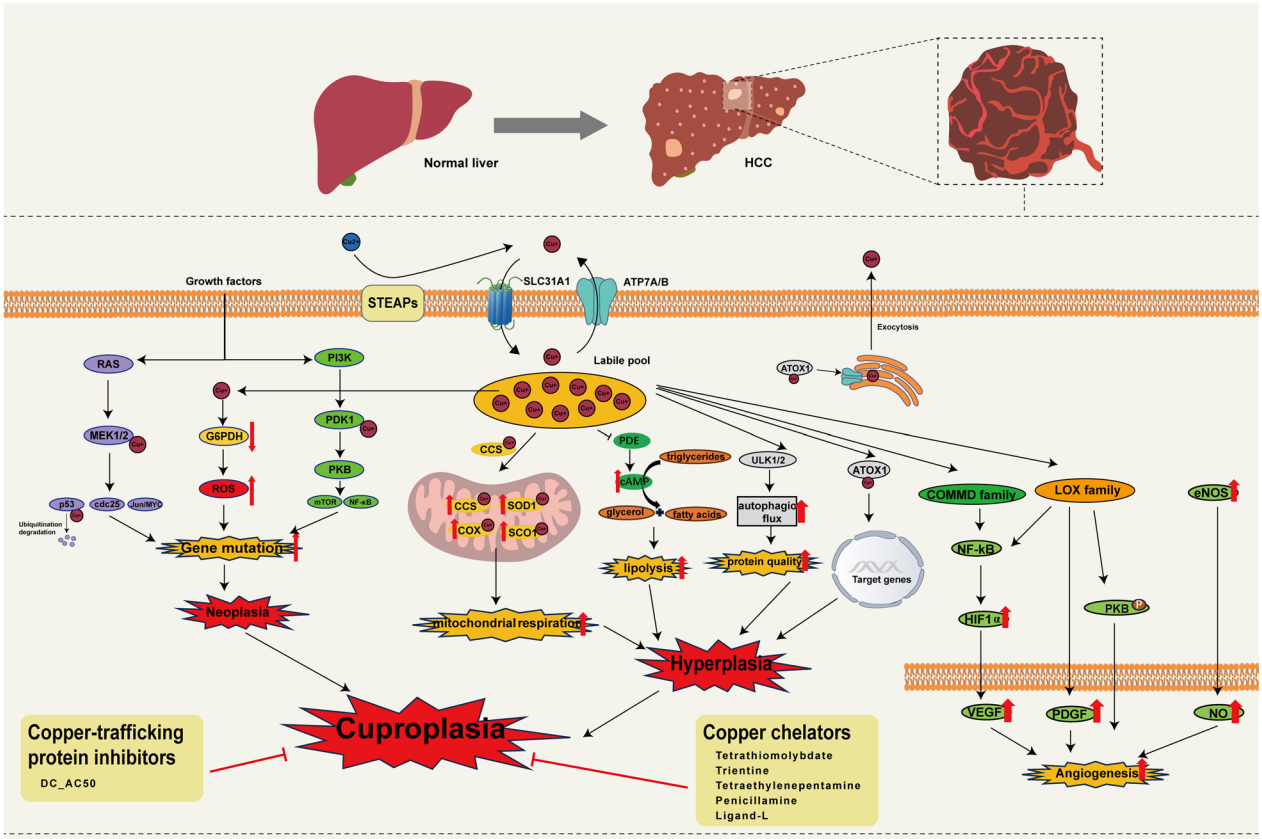

Fig. 1 The mechanism of cuproplasia in HCC. The cuproplasia is defined as the copper-dependent cell growth and proliferation, containing both neoplasia and hyperplasia. Copper is translated by the SLC31A1, and ATP7B and forms into the labile pool. (1) Copper could decrease the level of G6PDH and GRD, leading to the production of ROS and decreased antioxidant function, thus increasing the risk of genetic mutations in hepatocytes. Besides, through the BRAF-RAS-RAF-MEK-ERK and PI3K-PDK1-PKB signaling pathways, the risk of genetic mutations is increasing collectively in order to contribute to neoplasia. (2) Copper could promote hyperplasia through the mitochondrial pathways and non-mitochondrial pathways. Copper is the cofactor of various respiratory enzymes, the elevated copper could increase the number and the function of the respiratory enzymes in mitochondria such as the CCS, SOD1, COX, and ATOX1, thus the mitochondrial respiration is enhanced. Besides, copper could inhibit the PDE, which could degrade the cAMP. Thus, more triglycerides are transformed into glycerol and fatty acids through cAMP pathway, increasing the lipolysis in HCC, and consequently promoting tumor proliferation. (3) In addition, copper alleviates the ULK1 and ULK2 pathways to enhance autophagic flux, providing more copper-dependent targets for tumor proliferation by controlling protein quality. (4) Furthermore, copper is involved in the COMMD family and LOX family through HIF1α/VEGF/NF-κB pathway and ATOX–ATP7A–LOX pathways, then the angiogenesis is promoted. All of those above lead to hyperplasia. *ATOX1* antioxidant protein 1, *cAMP* 3′,5′-cyclic AMP, *cdc25* cell division cyclin25, *CCS* copper chaperone for superoxide dismutase, *COMMD* copper metabolism MURR1 domain, *COX* cytochrome c oxidase, *eNOS* endothelial nitric oxide synthase, *G6PDH* glucose 6-phosphate dehydrogenase, *LOX* lysyl oxidase, *MEK1/2* mitogen-activated protein kinase kinase 1/2, *HIF-1α* hypoxia-inducible factor-1α, *mTOR* mammalian target of rapamycin, *Nf-kB* Nuclear factor kappa-B, *NO* nitric oxide, *p53* transformation-related protein 53, *PDE* phosphodiesterase, *PDK1* 3-Phosphoinositide-dependent protein kinase 1, *PDGF* platelet-derived growth factor, *PI3K* phosphatidylinositol 3-kinase, *PKB* protein kinase B, *ROS* reactive oxygen species, *SCO1* synthesis of cytochrome c oxidase 1, *SOD* recombinant superoxide dismutase, *STEAPs* Six-transmembrane epithelial antigen of the prostate, *ULK* Unc-51 Like Autophagy Activating Kinase, *VEGF* vascular endothelial growth factor

domain (COMMD) family plays crucial roles in either promoting or inhibiting HCC hyperplasia [56]. Increased COMMD 7 expressions and the reduction of COMMD1 and COMMD10 expressions in HCC tissues could promote hyperplasia via Nuclear factor kappa-B signal pathways [56, 57]. Elevated COMMD 3 expression in HCC tissues could stimulate the angiogenesis through hypoxia-inducible factor-1α/ VEGF/nuclear factor kappa-B pathway [58].

Cuproplasia in TME of HCC

TME, the microenvironment surrounding the tumor cells, significantly influences the pathogenesis of HCC [59]. Apart from malignant hepatocytes, the TME of HCC encompasses surrounding extracellular matrix (ECM), innate and adaptive immune cells, inflammatory cells, Tumor-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), tumor-associated neutrophils, myeloid-derived suppressor cells (MDSCs),

endothelial cells, surrounding micro-vessels, and various cytokines and chemokines [60]. These cells interact with HCC, forming an immunosuppressive microenvironment [61, 62]. In addition, modified enzymes such as proteases, metabolic feedstocks, metabolites, exosomes, microparticles, and biophysical properties including adhesion and viscoelasticity are also important parts of TME.

The TME in HCC is marked by aberrant angiogenesis, immunosuppression, dysregulated ECM remodeling, and chronic inflammation, initiating tumorigenesis, growth, self-renewal, metastasis, and immune escape, while hindering various anti-tumor treatments [63, 64]. For instance, malignant hepatocytes secrete VEGF to create a tumor-promoting microenvironment, [65] while innate and acquired immune cells exhibit dual effects of tumor promotion and anti-tumor activities [8]. Tumor-infiltrating lymphocytes, comprising T cells, B cells, and others, are frontline defenders combating tumors with relentless attacks until pathogens are eradicated. However, anti-tumor responses of Tumor-infiltrating lymphocytes are hindered in tumor patients, impeding effective tumor eradication due to factors like cuproplasia induced by mild copper elevation, as discussed below.

T cells, pivotal in adaptive immunity and antitumor responses, consist of helper, cytotoxic, and memory subsets. Nonetheless, within the tumor microenvironment, these cells endure prolonged ischemia, hypoxia, and arginine deficiency, impairing their normal functions. For instance, microenvironmental lactate accumulation substantially hampers T cell proliferation, cytokines secretion, and cytotoxic activity [66]. Furthermore, studies have indicated that increased microenvironmental copper levels correlate positively with PD-L1 expression. PD-L1, a well-studied immune checkpoint, binds to PD1, inducing T cell apoptosis and exhaustion by inactivating downstream pathways like Ras/MAPK, PI3K/AKT, etc [67]. Zhou et al. reported similar findings, suggesting DSF/Cu upregulates PD-L1 expression by inhibiting poly (ADP-ribose) polymerase 1 (PARP1) activity and inactivating glycogen synthase kinase-3β (GSK3β). This concurrent upregulation of ligands and receptors for immune checkpoints likely enables HCC to evade T cell-mediated killing [68]. Although upregulation of ligands and receptors for immune checkpoints, such as anti-PD1 therapy, has been approved for HCC and generally have manageable side effects, some patients may struggle with tolerability, leading to potential treatment discontinuation or reduced efficacy [69]. Understanding individual tolerance levels and side-effect profiles is thus critical for optimizing therapy in HCC patients and enhancing responses to immune checkpoint inhibitors. In order to enhance the tolerability and efficacy of treatments, future research directions may include gaining a deeper understanding of the biology of immune checkpoints to improve current checkpoint blockade therapies and inform the development of the next generation of immunotherapies [70].

Macrophages, crucial TME components, form an immune barrier, engaging in antitumor activities through antigen phagocytosis and cytokine secretion like TNF-α. Moreover, macrophages present antigens to lymphocytes, bolstering adaptive immunity [71]. TAMs, a subtype of macrophages in TME, are abnormally activated, including the naïve macrophages (also known as M0 macrophages), M1 macrophages activated by interferon, and the M2 macrophages activated by anti-inflammatory factors [72]. M1 macrophages produce anti-tumor factors like TNF-α, whereas M2 macrophages with lower antigen-presenting ability produce tumor growth factors and angiogenic factors (such as IL-6, IL-10, VEGF) that promote tumor growth [72]. Excess copper could lead to significant suppression of macrophage function [73]. Given significantly elevated copper levels in both tumor tissue and serum of HCC patients compared to normal individuals, copper likely influences macrophage differentiation akin to other metabolites and cytokines, which promote M2 macrophage polarization [74].

CAFs are inhibitory intermediates in the TME that correlate with poor prognosis in HCC [75]. CAFs participate in ECM remodeling, [76] and release IL-6, FGFs, VEGF, hepatocyte growth factors, and other cytokines to recruit inflammatory and immune cells to affect the immune response process [77, 78]. Tumor-infiltrating natural killer (NK) cells, whose abundance correlates positively with overall survival, exhibit cytotoxicity through perforin, granzyme, Factor-related Apoptosis ligand, etc., while modulating immune responses via cytokines and chemokines secretion, along with antigen presentation [79]. Copper chelators have been observed to augment NK cell infiltration, suggesting elevated copper levels may impede NK cell survival, proliferation, recruitment, and cytotoxic activity. Thus, employing copper chelators could offer a novel and promising therapeutic strategy [80]. Additionally, diverse immune cells (e.g., B cells, neutrophils, MDSCs) represent crucial immunosuppressive elements in the TME [3]. These cells interact with HCC, shaping an immunosuppressive microenvironment [61, 62].

The anti-tumor activity of the immune cells relies on intact mitochondrial respiration [81]. Imbalance of copper homeostasis can impair immune response of immune cells to tumor cells [81]. Due to the significance of copper in mitochondrial metabolism, cuproplasia enhances the mitochondrial respiration of HCC cells, rendering them more resistant to immune cell elimination. Besides, in the COMMD family, the expression of COMMD2/3/10 is strongly associated with immune infiltration in HCC,

[82] especially M0 macrophages, and neutrophils, [83] promoting the occurrence and development of TME. The LOX family, as the copper-dependent enzyme, acts on the remodeling of structural ECM crosslinks, promoting TME formation in HCC [84]. Research showed that the LOX-like2 (LOXL2) and LOXL4 is highly expressed in HCC tissues [85, 86]. ECM induces the upregulation of hypoxia-inducible factor-1α through DSPK1/2-ERK1/2 pathway that further stimulates LOXL2 expression in TAMs [87]. CAFs could up-regulate the expressions of LOXL2 in HCC cells, while HCC could also up-regulate the expression of LOXL2 in CAFs. This forms a positive cycle to significantly promote TME and HCC invasion [86].

Furthermore, LOXL4 induces an immunosuppressive phenotype of macrophages, leading to upregulation of PD-L1 expression and further inhibiting CD8 + T cell function [88]. *Voli and his colleagues* first indicated that copper regulates PD-L1 expression, serving as a downstream target of intratumoral copper [28]. The significant function of PD-L1 in the suppressive TME has been fully discussed, and enormous drugs targeting PD-L1 have been developed [89]. Beyond being highly expressed on the surface of T lymphocytes, B lymphocytes, macrophages, and dendritic cells, PD-L1 is highly expressed in HCC cells [60]. PD-1/PD-L1 pathway induces T lymphocytes apoptosis and exhaustion, suppresses B lymphocytes activation, negatively affects the differentiation of T lymphocytes, and inhibits tumor-specific T cell proliferation, [90] thus promoting immune tolerance and suppressive TME. The research found that copper chelators reducing the copper in tumor tissues could significantly decrease the expression of PD-L1, thereby increasing the tumor-infiltrating NK cells and $CD8^+$ T cells to promote new immune cell clones and enhance the anti-tumor immune responses, indicating the inhibitory effect of copper on TME [28].

Moreover, cuproplasia in TME activates HCC cells to secret more proangiogenic factors such as basic fibroblast growth factors, VEGF, and fibroblast growth factor 2, 3-phosphoinositide dependent protein kinase 1, enhancing migration and invasion of endothelial cells, thereby promoting angiogenesis [49, 62, 91]. Angiogenesis is an important factor in tumor progression. LOX family also promotes peritumoral angiogenesis by upregulating the expression of VEGF and platelet-derived growth factors [88, 92]. Through the ATOX1–ATP7A–LOX pathways, the LOX family promotes the distant metastasis of HCC [41]. Copper in TME also activates copper-related pathways such as the MAPK pathway, [48] the apyrimidinic endonuclease-1/redox effector factor 1 to promote HCC tumorigenesis and metastasis [93]. Studies have shown that Cu^{2+} could bind with CD147 to activate the PI3K-PKB pathway, stimulating surrounding fibroblasts to highly express angiogenic activators like matrix metalloproteinase 2, thus increasing HCC invasiveness [12].

Cuproptosis and HCC

The mechanism of cuproptosis

Cuproptosis is a currently discovered form of programmed cell death, distinct from known mechanisms triggered by copper and dependent on mitochondrial respiration [16]. The current understanding of the cuproptosis mechanism is depicted in Fig. 2. However, the more precise mechanisms require further investigation.

Copper, a key element in mitochondria, is involved in the assembly of copper enzymes such as COX, antioxidant enzyme superoxide dismutase 1, and respiratory complex IV [43]. The onset of cuproptosis relies on mitochondrial respiration and is closely related to the tricarboxylic acid (TCA) cycle [16]. Normal cells regulate intracellular copper through copper importer SLC31A1 and exporter ATP7B [94]. Both transporters are strongly related to cuproptosis [95]. When the level of copper in ECM is elevated, elesclomol, a copper ionophore, transports copper into the intracellular matrix [96]. Elesclomol enhances the ferredoxin 1 (FDX1), a reductase capable of reducing Cu^{2+} to more toxic Cu^{+} [97]. Additionally, FDX1 disrupts Fe-S protein balance and promotes lipidation and aggregation of enzymes involved in the regulation of the TCA cycle, especially dihydrolipoamide S-acetyltransferase (DLAT) [98]. Excess Cu^{+} induces proteotoxic stress rather than copper-induced mitochondrial oxidative stress by causing mitochondrial lipidated protein oligomerization [16]. Moreover, cuproptosis induction impairs mitochondrial respiration, resulting in reduced ATP synthesis. ATP depletion activates Adenosine 5'-monophosphate(AMP)-activated protein kinase (AMPK), triggering the phosphorylation of high-mobility group box 1 (HMGB1) [99]. Under normal circumstances, HMGB1 primarily resides in the nucleus, tightly bound to nucleosomes [100]. Upon AMPK-induced phosphorylation, HMGB1 dissociates from histones, leading to increased extracellular release, inflammation, [99] exacerbation of cell death, and tissue damage (Fig. 3).

Cuproptosis in HCC

Cuproptosis is triggered by abnormal expression of cuproptosis-related genes (CRGs) [101]. Numerous CRGs have been identified in HCC involving in copper ion metabolism and mitochondrial function (Table 1). Sun et al. found that FDX1 downregulation activates mitophagy and the PI3K/AKT signaling pathway, promoting HCC progression through elevated ROS production [102]. DLAT was overexpressed in HCC tissues and promotes HCC cell proliferation [103]. Lipoic acid

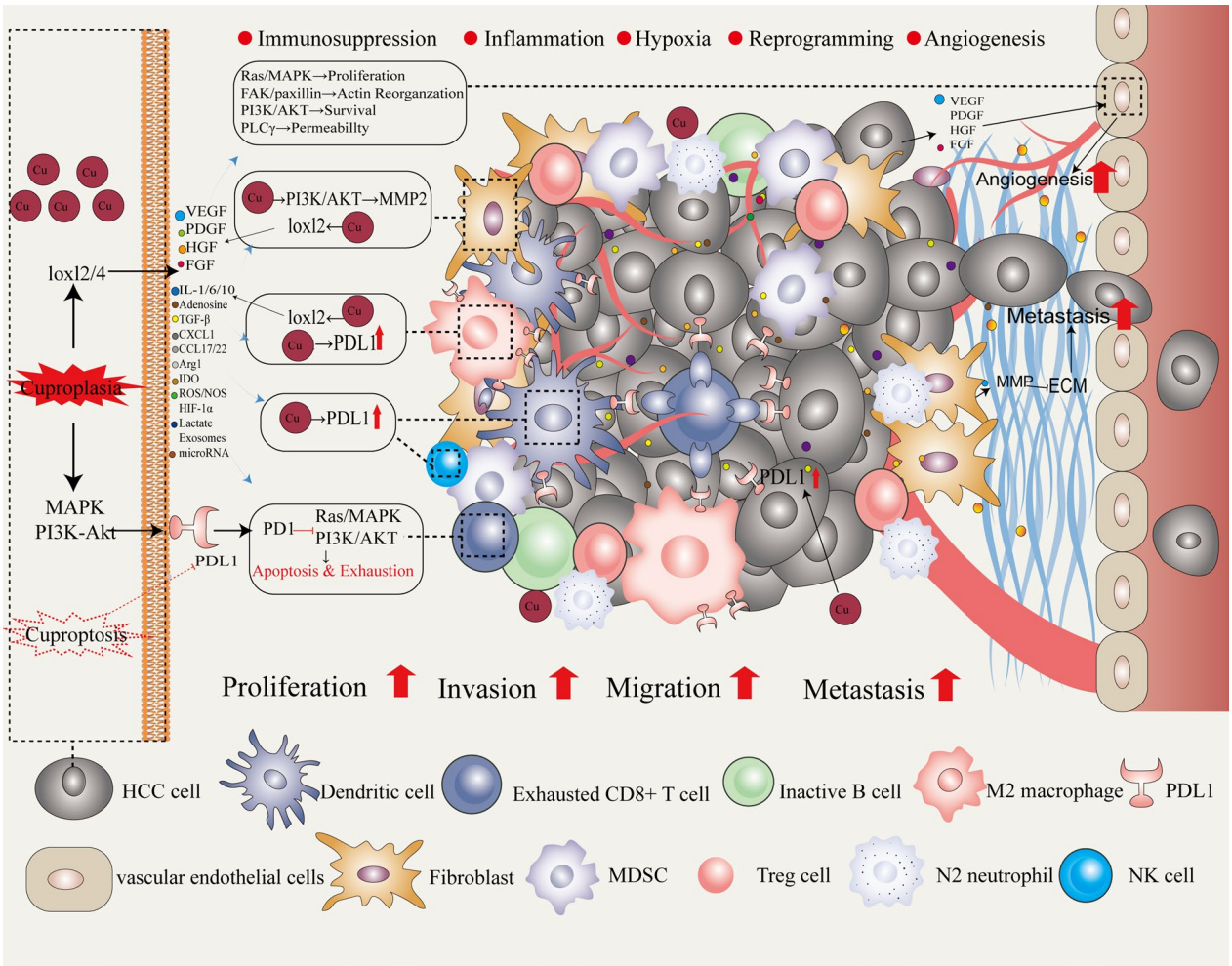

Fig. 2 Cuproplasia in TME of HCC. The TME in HCC is marked by aberrant angiogenesis, immunosuppression, dysregulated ECM remodeling, hypoxia, reprogramming, and chronic inflammation, initiating tumorigenesis, growth, self-renewal, metastasis, and immune escape. Cuproplasia induces high expression of LOX2 or LOX4 in HCC cells, CAFs, and TAMs, leading to the release of factors such as VEGF, PDGF, HGF, and FGF. These factors promote endothelial cell survival, proliferation, and angiogenesis, while also upregulating MMP2 expression by CAFs, thereby enhancing HCC invasiveness. Furthermore, cuproplasia upregulates PDL1 expression in HCC cells, CAFs, TAMs, and NK cells, resulting in apoptosis and exhaustion of CD8+T cells. Alongside other cytokines, chemokines, metabolic substances, ROS, exosomes, etc., an immunosuppressive TME ultimately develops. *CAFs* cancer-associated fibroblasts, *ECM* extracellular matrix, *FAK* focal adhesion kinase, *FGF* fibroblast growth factor, *HCC* hepatocellular carcinoma, *HGF* hepatocyte growth factor, *LOX2* lysyl oxidase-like2, *LOX4* lysyl oxidase-like4, *MAPK* Mitogen-Activated Protein Kinase, *MMP2* Matrix metalloproteinase 2, *NK cells* natural killer cells, *PI3K* phosphatidylinositol 3-kinase, *PDGF* platelet-derived growth factor, *PDL1* programmed death-ligand 1, *RAS* rat sarcoma, *ROS* reactive oxygen species, *TAMs* Tumor-associated macrophages, *TAN* tumor-associated neutrophils, *TME* tumor microenvironment, *VEGF* vascular endothelial growth factor

significantly inhibited cell migration and invasion in HCC cells [104]. These genes contribute to regulating copper-dependent cell death mechanisms, which could influence HCC progression. Additionally, anti-cuproptosis genes (e.g., GLS, MTF1, CDKN2A) may be linked to antioxidant functions, stemness formation, angiogenesis, DNA repair, and methylation [105–107]. Copper transporters (e.g., SLC31A1, ATP7B) enable HCC cells to counteract the cytotoxic effects of copper buildup, allowing tumor cells to evade apoptosis [108]. For additional information on CRGs' roles and mechanisms in HCC development, refer to Table 1. These findings indicate that targeting CRGs and copper homeostasis may offer new therapeutic strategies for HCC. Further research is needed to clarify these pathways and their effects on HCC treatment outcomes.

Among the CRGs, 7 pro-cuproptosis genes (FDX1, DLAT, lipoic acid synthetase gene, lipoyltransferase 1, dihydrolipoamide dehydrogenase gene, pyruvate dehydrogenase E1 subunit beta gene, and pyruvate

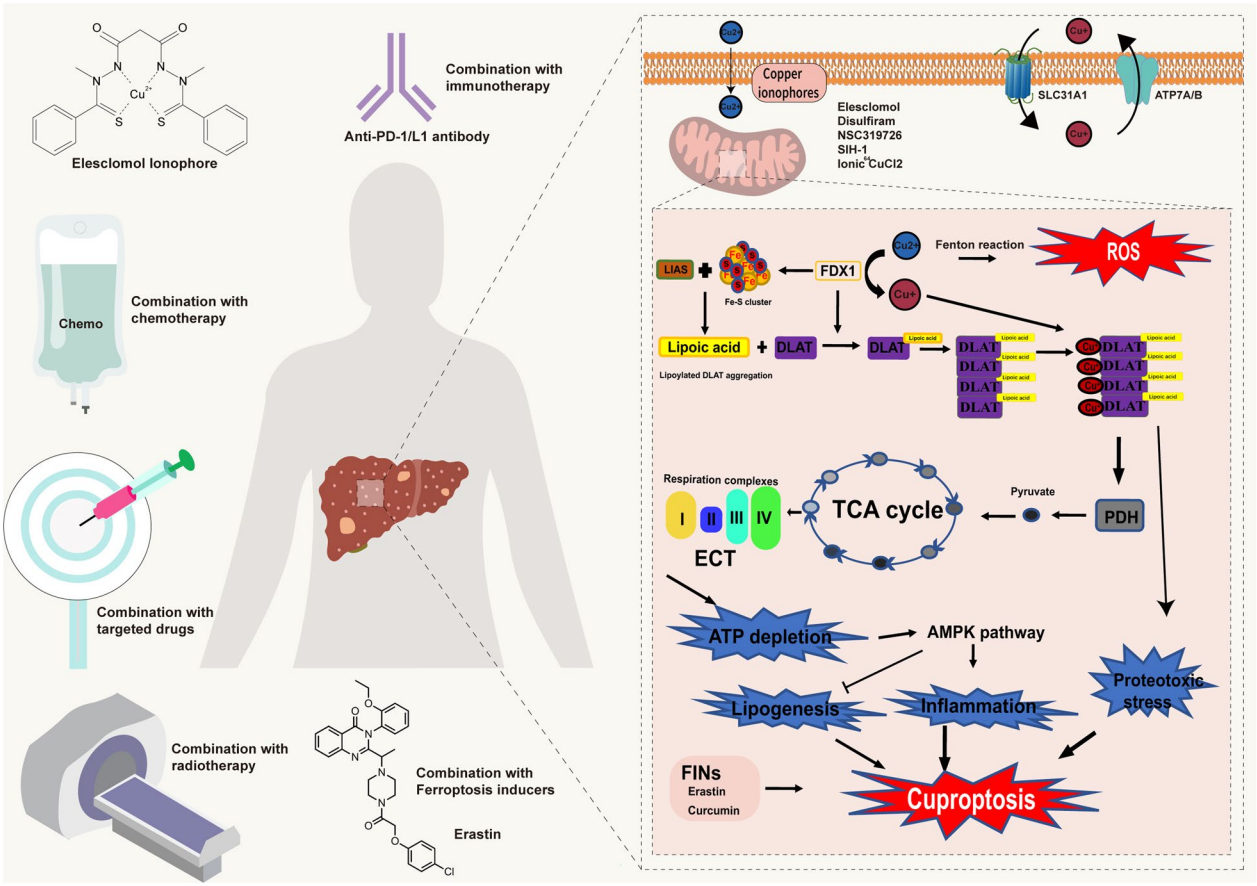

Fig. 3 Cuproptosis and its mechanism in HCC. The cuproptosis is closely related to mitochondrial respiration and the TCA cycle and is triggered by the elesclomol, which could translate elevated extracellular copper to intracellular matrix not only the cell membrane importer SLC31A1 and exporter ATP7B. Besides, elesclomol could enhance the FDX1 to reduce Cu^{2+} to become the more toxic Cu^{+}. FDX1 is the key enzyme of cuproptosis, causing Fe-S protein imbalance thus leading to the Fe-S cluster, and promoting the lipoylation of DLAT. The Cu + binds to the lipoylated DLAT aggregation to participate in the formation of the PDH complex, which could affect the mitochondrial TCA cycle, leading to ATP depletion. Depletion of ATP will lead to intracellular inflammation through the AMPK pathway. In addition, the lipoylated DLAT aggregation and excess $Cu.^{+}$ could induce proteotoxic stress through mitochondrial lipidated protein oligomerization. Eventually, these serious consequences lead to the cuproptosis. *AMPK* Adenosine 5′-monophosphate(AMP)-activated protein kinase, *ATP* Adenosine 5′-triphosphate, *ATP7B* Adenosine 5′-triphosphatase copper transporting beta, *DLAT* dihydrolipoamide S-acetyltransferase, *ECT* electron transport chain, *FDX1* ferredoxin 1, *LIAS* lipoic acid synthetase, *TCA* tricarboxylic acid, *PDH* Pyruvate dehydrogenase, *SLC31A1* solute carrier family 31 member 1

dehydrogenase E1 subunit alpha 1 gene), 3 anti-cuproptosis genes (glutaminase gene, metal regulatory transcription factor 1 gene, and cyclin-dependent kinase inhibitor 2A gene), and 2 transporters (SLC31A1 and ATP3B) are involved in the process of cuproptosis [95]. Unlike apoptosis, mitochondrial ROS, B-cell lymphoma-2-associated X protein, B-cell lymphoma-2-antagonist/killer 1, and caspase activation required for apoptosis are not required for cuproptosis, [16] despite the decrease in mitochondrial membrane potential [16, 99]. Oxidative stress inhibitors (e.g., N-acetylcysteine), ferroptosis inhibitors (e.g., ferrostatin-1), or cell necrosis inhibitors (e.g., necrostatin-1) cannot inhibit cuproptosis. However, copper chelators, inhibitors of respiratory chain complex I (Rotenone), II (Rustin), and III (antimycin A), and inhibitors of mitochondrial pyruvate uptake (UK5099) can inhibit cuproptosis [16]. Cuproptosis is unaffected by mitochondrial uncouplers like Synonyms of Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone, suggesting that mitochondrial respiration rather than ATP production is required for cuproptosis [16]. The mitochondrial quality control systems like mitophagy, and AMPK-mediated autophagy pathway could promote mitochondrial health and homeostasis. Mitophagy could self-repair mitochondria to enhance respiratory function under stressful situations, [109] indicating that activating those mitochondrial quality control systems may limit cuproptosis [98, 109].

Table 1 The CRGs and their developed scoring methods in HCC

Researchers	CRGs	The Highlights	References
Zhen Zhang and his colleagues	FDX1 and related genes(ALDH5A1, CAT, EHHADH, and SLC27A)	CRRS: The high CRRS group showed lower survival and increased tumor immune infiltration	[10]
Lei Ding and his colleagues	CDKN2A, DLAT, DLD, FDX1, GLS, LIAS, LIPT1, MTF1, PDHA1, and PDHB	1. Three subtypes of cuproptosis were validated; 2. CRGPI: could be used as a potential biomarker for prognosis and immunotherapy in HCC patients	[112]
Zhiqiang Liu and his colleagues	ATP7A, ATP7B, DBT, DLAT, DLD, DLST, FDX1, GCSH, LIAS, LIPT1, PDHA1, PDHB, and SLC31A1	1. Three subgroups of patients based on CRGs were revealed; 2. A cuproptosis-related prognostic risk model was built to predict prognosis	[113]
Jie Fu and his colleagues	ATP7A, ATP7B, CDKN2A, DLAT, DLD, FDX1, GCSH, GLS, LIAS, LIPT1, LIPT2, MPC1, MTF1, PDHA1, PDHB, SLC31A1	1. Three distinct CRGs expression patterns were identified; 2. CRRS was established to predict the prognosis, the immune microenvironment, and expression of immune checkpoint molecules	[111]
Tianhao Cong and his colleagues	Cuproptosis-related ICGs (BTN2A1, BTNL9, CD276, CD40LG, LGALS9, SIRPA, TNFRSF4)	Cuproptosis-related ICGs were developed to predict the prognosis and immune response of HCC patients	[114]
Xi Chen and his colleagues	ATP7A, ATP7B, BAD, PDHA1, CCS, CDKN2A, DLAT, DLD, FDX1, GLS, LIAS, LIPT1, MTF1, MTOR, NRF2, PDHB, and SLC31A1	1. Three cuproptosis subtypes were identified; 2. Cuproptosis signature has been built containing five cuproptosis-associated genes: CLEC3B, CFH, HPR, LAMB1, and PFKFB3	[101]

ALDH5A1 aldehyde dehydrogenase 5 family member A1, *ATP7A* ATPase copper transporting alpha, *ATP7B* ATPase copper transporting beta, *BAD* BCL2 associated agonist of cell death, *BTNL9* butyrephilin like 9, *BTN2A1* butrophilin subfamily 2 member A1, *CAT* catalase, *CCS* copper chaperone for superoxide dismutase, *CD40LG* CD40 antigen ligand, *CDKN2A* cyclin dependent kinase inhibitor 2A, *CRG* cuproptosis-related gene, *CRRS* cuproptosis-related risk score, *CRGPI* cuproptosis-related gene prognostic index, *DBT* dihydrolipoamide branched chain transacylase E2, *DLAT* dihydrolipoamide S-acetyltransferase, *DLST* dihydrolipoamide S-succinyltransferase, *DLD* dihydrolipoamide dehydrogenase, *EHHADH* enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase, *FDX1* ferredoxin 1, *GCSH* glycine cleavage system protein H, *GLS* glutaminase, *ICG* immune checkpoint genes, *LIAS* lipoic acid synthetase, *LGALS9* galectin 9, *SLC27A* solute carrier family 27A, *LIPT1* lipoyltransferase 1, *MTF1* metal regulatory transcription factor 1, *MTOR* mammalian target of rapamycin, *MPC1* mitochondrial pyruvate carrier 1, *NRF2* Nuclear factor erythroid2-related factor 2, *PDHA1* pyruvate dehydrogenase E1 subunit alpha 1, *PDHB* pynuvate dehydrogenase E1 subunit beta, *SIRPA* signal regulatory protein alpha, *SLC31A1* solute carrier family 31 member 1, *TNFRSF4* TNF receptor superfamily member 4

The dysregulation of CRGs is closely related to the occurrence and development of HCC, [101, 110–113] and CRGs expressions strongly correlate with the immune-suppressive TME in HCC [111, 114]. CRGs enable the identification of HCC subtypes (Table 1). Altered expression of CRGs such as SLC31A1 and ATP7B is significantly associated with elevated copper levels in HCC tissues [33]. Multi-omics analysis reveals that CRGs could identify HCC patients with suppressed immune TME, increased expression of immune checkpoint molecules, and poor prognosis [111]. CRG scores inversely correlate with the expression of immune-related genes and positively correlate with the expression of DNA repair-related genes [115]. A bioinformatics analysis results showed that CRG score was significantly correlated with the infiltration of M2 macrophages and neutrophils in TME [116]. M2 macrophages induce hypoxia and suppress the immune system, [117] while neutrophils secrete chemokine ligands that mediate the infiltration of M2 macrophages and regulatory T cells, reshaping the TME to suppress the immune response [118].

CRGs were expressed to varying degrees in all cell types of TME [111]. For instance, SLC31A1 is positively correlated with the abundance of M1 macrophages and neutrophils, while FDX1 positively correlates with the abundance of activated memory $CD4^+$ T cells [111, 114]. Some cuproptosis-activating genes (e.g., lipoic acid synthetase gene, lipoyltransferase 1, FDX1, and pyruvate dehydrogenase E1 subunit alpha 1 gene) exhibit resistance to drugs with broad anti-inflammatory activity, speculating the similar anti-inflammatory biological activities of those genes, consistent with the suppressed immune infiltration [115]. High expression of DLAT in cancer tissues enables HCC cells to evade the immune system and foster an inhibitory immune microenvironment [119]. The proportion of Tregs among HCC patients with long overall survival is higher than that among those with shorter survival, [110] partly due to Tregs' involvement in regulating cuproptosis-induced inflammatory responses and oxidative stress in the TME [110].

Cuproptosis is speculated to shape the antitumor immune environment, but its inhibitory effect on immunotherapy remains uncertain. [120] Previous research showed that various immune checkpoint genes (ICGs) such as butyrophilin subfamily 2 member A1 gene, Butyrophilin-like protein 9, CD40 antigen ligand, signal regulatory protein alpha gene, and TNF receptor superfamily

member 4 gene are co-expressed with CRGs [114]. High expression of CRGs is positively correlated with the immune checkpoint molecules [111]. ICGs are closely related to the incidence and development of HCC, which forms the basis of immunotherapy and immune checkpoint inhibitor (ICI) therapy [121]. Additionally, the CRGs are closely associated with the immunosuppressive TME, which is also an essential indicator of ICI therapy. Therefore, CRGs affect the immune microenvironment and are throughout the development of HCC, which is closely related to immunotherapy.

HCC cells exhibit both aerobic respiration (oxidative phosphorylation) and glycolysis, with a preference for glycolysis, a phenomenon known as the Warburg effect [122]. This metabolic characteristic suggests that HCC proliferation is characterized by increased glycolysis even under the presence of O_2 [123]. Besides, HCC thrives in a hypoxic TME, further promoting glycolysis [61]. The increased glycolysis is closely related to the proliferation, angiogenesis, and metastasis of HCC [124, 125]. Thus, inhibiting glycolysis or reversing the hypoxic TME can potentially delay HCC progression [126]. Cuproptosis is closely related to mitochondrial respiration, mainly involving oxidative phosphorylation [16]. The occurrence of cuproptosis requires enhanced oxidative phosphorylation level and improved aerobic TME. Therefore, promoting cuproptosis in HCC may alleviate mitochondrial dysfunction and hypoxic TME, thus reducing the glycolysis in HCC cells, offering a fascinating perspective for clinical treatment [127].

The relationship between cuproplasia and cuproptosis

The occurrence of cuproplasia or cuproptosis in a cell is contingent upon the concentration of copper ions. Slightly increased copper concentrations not only sustain cellular functions and fulfill physiological and metabolic needs but also contribute to tumorigenesis, as previously elucidated in detail, resulting in cuproplasia [9]. Conversely, surpassing a specific threshold of copper leads to cuproptosis, apoptosis, ferroptosis, and other cellular responses [27]. Nonetheless, cells exhibit varying degrees of copper tolerance. Studies have revealed notably heightened copper levels in diverse tumor tissues, indicating enhanced capacity of tumor cells in copper utilization and tolerance [80]. This could be attributed to the maximal utilization of cuproplasia by tumor cells to proliferate via the aforementioned mechanisms, along with heightened autophagy aimed at clearing ROS produced by the elevated copper-induced Fenton reaction, thereby shielding tumor cells from cytotoxic effects of uncontrolled peroxidation. However, genes linked to these mechanisms could be either downregulated or upregulated in anti-tumor immune cells. For instance, autophagy plays a crucial role in sustaining T cell proliferation and function. However, tumor-infiltrating T cells exhibited notable downregulation of autophagy-related genes with decreased autophagic flux, leading to inhibition of T cell proliferation and oxidative stress [128].

The autophagy pathway plays a crucial role in connecting the mechanisms of both cuproplasia and cuproptosis. Lower copper levels can alleviate the ULK1 and ULK2 pathways, thereby enhancing autophagic flux, regulating protein quality, and ultimately promoting copper-dependent tumor proliferation. Additionally, autophagy could be induced by metabolic stress, hypoxia, redox stress, and immune signaling such as damage-associated molecular patterns [129]. Upregulated autophagy in tumor cells can facilitate tumorigenesis and progression by reducing ROS accumulation and providing essential nutrients for survival. Autophagy also contributes to the degradation of granzyme B released by NK cells and cytotoxic T lymphocytes, as well as major histocompatibility complex class I in dendritic cells, promoting tumor immune evasion [130]. Furthermore, chemoresistance and distant metastasis in HCC could be attributed to enhanced autophagy, which decreases the sensitivity to chemotherapy and inhibits anoikis [130].

However, the disruption of the mitochondrial TCA cycle by excess copper leads to ATP depletion, triggering intracellular inflammation and increased autophagy via the AMPK pathway, exacerbating the process of cuproptosis. Therefore, autophagy acts as a double-edged sword in tumors, with its specific role determined by the specific tumor type, differentiation degree, pathological stage, and the TME context. Elesclomol–CuCl2 can mitigate resistance to docetaxel by inhibiting autophagy in prostate cancer cells [131]. In colorectal cancer models, tretinoin demonstrates antitumor effects by inhibiting both proliferation and autophagy [132]. Although direct evidence is lacking for the involvement of autophagy inhibition in cuproplasia or cuproptosis in HCC, the role played by inhibition of autophagy in other tumors presents suggestive testimony. ROS mediates the processes of cuproplasia and cuproptosis. Copper may reduce G6PDH and GRD levels, resulting in ROS production and decreased antioxidant function. This increases the risk of genetic mutations in hepatocytes, thereby promoting neoplasia [133]. Copper also enhances the activity of copper chaperone for superoxide dismutase and SOD1, which convert superoxide, with high activity, into hydrogen peroxide. This reduces ROS production in the mitochondrial membrane and mitigates ROS-induced damage to tumor cell proteins and lipid membranes [134]. Moreover, ROS increases autophagic flux, thereby offering additional copper-dependent targets for tumor

proliferation by regulating protein quality [22]. MDSCs, TAMs, and neutrophils exert immunosuppressive functions through various pathways such as Treg induction, production of high levels of arginase-1 and ROS. Copper chelator usage substantially decreased MDSCs infiltration, indicating a proportional relationship between copper levels and MDSCs recruitment [80]. Elevated copper levels promote increased MDSCs and ROS levels, creating an immunosuppressive microenvironment that mediates immune evasion. Mitochondrial Fe-S cluster proteins, involved in the synthesis of heme, lipoic acid, and biotin in mitochondria, are crucial constituents of the TCA cycle and the electron transport chain complexes [135]. Copper cytotoxicity results in Fe-S cluster loss, leading to reduced mitochondrial membrane potential, inhibition of the electron transport chain complexes and TCA cycle, and initiation of the Fenton reaction, resulting in substantial ROS production. ROS burst exacerbates cuproptosis by inducing multifaceted cellular damage, including DNA damage, mitochondrial dysfunction, and membrane integrity disruption.

The clinical significance and prospect of cuproplasia and cuproptosis in HCC

Copper compounds and nano-copper

Copper induces both cuproplasia and cuproptosis in HCC. Tumor cells need more copper than normal cells for proliferation, angiogenesis, and metastasis, [43] while excessive copper also triggers cuproptosis. Thus, inhibiting cuproplasia and enhancing cuproptosis in HCC could be prospective therapeutic strategies. Cellular fate, whether cuproplasia or cuproptosis, is determined by the level of copper concentration. Consequently, targeting copper via copper chelators to mitigate cuproplasia or copper ionophores to enhance cuproptosis has emerged as a highly promising therapeutic avenue for various copper-associated diseases, including HCC. Identifying the multifaceted roles of crucial molecules involved in autophagy and ROS regulation in cuproplasia and cuproptosis, along with potential targets, agents, and combined therapeutic interventions utilizing copper compounds, may yield more efficacious therapeutic strategies. Copper compounds, appropriately complexed, hold promise as potential drugs for HCC treatment with minimal side effects [27].

Clinically used copper compounds include copper chelators and copper ionophores, both exhibiting outstanding anticancer activity and promising prospects in cancer therapy (Table 2) [91]. Copper chelators inhibit cuproplasia in tumor cells, contributing to therapeutic efficacy [32]. Numerous copper chelators have been developed at present, [136] and studies have demonstrated their ability to inhibit the formation of new blood vessels, thereby preventing angiogenesis [52, 137]. Tetrathiomolybdate (TTM), a copper chelator capable of inhibiting copper absorption, has demonstrated efficacy in reducing the tumorigenicity of HCC cell lines. It also inhibits glycolysis, reducing the energy supply to tumor cells, and thereby impeding tumor initiation and progression [33]. Given that the occurrence and development of HCC are closely linked to angiogenesis, there has been a surge in research aiming to inhibit this process, leading to advancements in anti-HCC therapies focused on antiangiogenesis. Penicillamine, known for its role as an antidote for heavy metal poisoning and in treating Wilson's disease, has emerged as a potential anti-HCC drug due to its strong copper chelating properties and anti-angiogenic effects [138]. Trientine, an alternative for patients with Wilson's disease who cannot tolerate penicillamine, is another effective copper chelator that inhibits angiogenesis. Sone K et al. reported that trientine not only inhibits the proliferation of vascular endothelial cells but also promotes tumor cell apoptosis, exhibiting a remarkable and promising anti-tumor activity [139]. Besides, copper chelators could reduce PD-L1 expression in tumor tissue, stimulate anticancer immune responses, and inhibit immune checkpoints [28]. In addition, pro-chelators are developed to enhance selectivity against cancer cells [140]. By utilizing stimuli primarily present in the TME, pro-chelators enhance targeting activity and therapeutic effects with little off-target toxicity [136, 141]. Regarding copper ionophores, research found that in some cases there may be a critical copper solubility and a narrow window that enables more copper to accumulate in HCC tumor cells leading to cuproptosis and thus selectively killing tumor cells [15]. Disulfiram, a widely studied copper ionophore, can inhibit the activity of PARP1, promote the phosphorylation of GSK-3β at the Ser9 site, and ultimately lead to the increase of PD-L1 expression and stimulate cell apoptosis [68]. Elesclomol, a highly lipophilic and potent copper-binding molecule, transports excess copper into mitochondria, causing loss of lipoylated mitochondrial proteins and Fe-S cluster protein, triggering intense oxidative stress, ultimately leading to cuproptosis in HCC cells [142].

To enhance the selectivity of copper ionophores, pro-ionophores and nano-drug delivery systems can be used as *Valentina Oliveri and her colleagues* detailed in their review [32]. Nano-copper is a popular potential anti-tumor drug for chemodynamic therapy recently, [143] which regulates the immunosuppressive TME by activating ROS to kill tumor cells and alleviate the hypoxic microenvironment to trigger immunogenic cell death [144]. GSH is overexpressed in the TME and severely depletes ROS to limit the chemodynamic therapy [145]. Studies have found that Cu^{2+} released by nano-copper

Table 2 Drugs or medicines for HCC according to the copper mechanism

Researchers	Types of Medicine	Medicines	Mechanisms		References
Caroline I Davis et al	**Copper chelators**	**Tetrathiomolybdate(TTM)**	**Cuproplasia**	**TTM can reduce the tumorigenicity of HCC cell lines as well as inhibit glycolysis to reduce the energy supply to tumor cells**	**[33]**
Sone K.; Yoshii J. et al		Trientine		Trientine can suppress neovascularization, inhibit endothelial cell proliferation, and promote cell apoptosis	[138, 139]
Yoshii J. et al		Penicillamine		Penicillamine can inhibit angiogenesis to suppress the development of HCC	[138]
Mi Yang et al		Tetravinylpentylamine		Tetravinylpentylamine resensitizes the tumor cells to radiation in mice-fed copper	[159]
Saman Khan et al		ligand-L		ligand-L induces ROS production, causing DNA, protein, and lipid damage, and promotes HCC cell death	[173]
Zhou B. et al	Copper ionophores	Disulfiram (DSF)	Cuproptosis	DSF/Cu can inhibit the activity of PARP1, promote the phosphorylation of GSK-3β at the Ser9 site, and ultimately lead to the increase of PD-L1 expression and stimulate cell apoptosis	[68]
Gao F. et al.; Li D. et al		Elesclomol		Elesclomol can induce cuproptosis in HCC cells while causing the loss of lipoylated mitochondrial proteins and Fe-S cluster protein	[142, 174]
Wachsmann J. et al		Ionic [63] $CuCl_2$		hctr1 gene expression is often upregulated in HCC cells, where Ionic [63]$CuCl_2$ can be used in radiation therapy	[52]
Yuan Ji et al		SIH-1		SIH-1 releases copper through GSH, causing redox imbalance and mitochondria-mediated apoptosis in HepG2 cells	[175]
Tianxiu Dong et al	Nano-copper	PFP@PLGA/Cu12Sb4S13 nanocapsule (PPCu)	Cuproptosis	PPCu can inhibit the RAS/MAPK/MT-CO1 signaling pathway, normal mitochondrial function, and promote apoptosis of hepatocellular carcinoma cells	[176]
Jean-Pascal Piret et al		Copper oxide nanoparticles (CuONPs)		CuO NPs can stimulate human hepatocellular carcinoma HepG2 cells to produce ROS, activate AP-1, as well as activate MAPK, ERKs, and JNK/SAPK signaling pathways	[177]
Siddiqui et al				CuONPs can play an antitumor role in Hep G2 cells by up-regulating caspase-3 gene expression	[47]
Zheng Yang et al		Au25(NAMB)18 NCs-Cu2 + @SA/HA NHGsC		Au25(NAMB)18 NCs-Cu2 + @SA/HA NHGsC possesses targeted and NIR laser-responsive properties and depletes overexpressed GSH and H2O2 in the TME. Its imaging properties enable image-guided diagnosis and treatment of tumors	[178]

AP-1 activator protein-1, *ERK* extracellular regulated protein kinases, *GSK-3β* glycogen synthase kinase 3β, *hctr1* human copper transporter 1, *JNK* c-Jun N-terminal kinase, *ligand-L* di(2-picolyl)amine-3(bromoacetyl)coumarin hybrid molecule, *MAPK* Mitogen-Activated Protein Kinase, *MT-CO1* mitochondrially encoded cytochrome c oxidase I Gene, *PARP1* poly (ADP-ribose) polymerase 1, *PD-1* programmed cell death protein 1, *RAS* rat sarcoma, *SAPK* stress-activated protein kinase, *SIH-1* salicylaldehyde isonicotinoyl hydrazone

can effectively consume the overexpressed GSH in TME, thereby reducing the elimination of hydroxyl radicals and amplifying cascade oxidative stress [143]. In addition, the generated Cu^{+} utilizes the characteristics of TME to trigger nano-catalysis reactions via Fenton-like reactions, [146] producing highly toxic hydroxyl radicals and hydrogen peroxide, leading to the outbreak of ROS in the TME [147]. High concentrations of ROS induce severe oxidative stress thus triggering immunogenic cell death. During the immunogenic cell death, the antigen-presenting cells and cytotoxic T lymphocytes are activated and the systemic anti-tumor immune responses are triggered [144]. In addition, to enhance the therapeutic effect, enormous materials such as copper nanocrystalline-doped folic acid-based super carbon dots, [145] copper-encapsulating magnetic nanoassemblies, [148] thermosensitive hydrogel systems, and copper-coordinated nanogenerators were developed [143, 147]. Although success has been achieved in preclinical research, clinical applicability still needs further research.

DC_AC50, another copper-based anticancer compound, acts as a copper-trafficking protein inhibitor, inhibiting ATOX1/copper chaperone for superoxide dismutase to enhance ROS-mediated cell death in lung cancer H1299 cells, head and neck cancer 212LN cells, and breast cancer cells [134]. Preclinical studies in melanoma patients have revealed the significant therapeutic potential of DC_AC50, offering suggestive evidence for copper-targeted therapy in HCC [149].

Combination therapy

Combination with targeted drugs. Lenvatinib, a receptor tyrosine kinase inhibitor that suppresses vascular endothelial growth factor receptors, has already widely been put into clinical applications in advanced HCC. However, the therapeutic effects of Lenvatinib are still subject to certain limitations such as the rapid development of drug resistance and side effects. In 2021, *Qi Xu* and co-workers constructed a nano platform where Lenvatinib and copper sulfide nanocrystals (Cu2-xS NCs) were co-encapsulated [150]. The evidence from this study indicated that the combinatorial treatment enhanced tumoricidal efficacy and provided additional therapeutic benefits. Similarly, surveys conducted by *Li Nan* et al. in 2023 conclusively suggested that the application of a combination of TTM with Lenvatinib presented markedly decreased angiogenesis and showed synergistic antitumor responses [151]. Sorafenib, another widely studied receptor tyrosine kinase inhibitor, exhibits a significant advantage in suppressing angiogenesis and tumor cell proliferation, extending the survival time of advanced patients [152]. Mechanistically, sorafenib inhibits mitochondrial matrix-related proteases-mediated FDX1 degradation and directs tumor cells to cuproptosis [153]. Wang and colleagues found that disulfiram (a copper ionophore mentioned above) combined with sorafenib has significant synergistic cytotoxicity against neoplastic cells of the liver and shows extremely encouraging anticancer and anti-metastasis efficacy. Collectively, these results provide novel insights into combination treatment strategies in HCC [154].

Combination with chemotherapy and radiotherapy. *Li* et al. published the findings of the prediction response of 10 drugs in hepatocellular carcinoma. Among them, they found that patients with a high CRGs score subtype were more sensitive to 5-fluorouracil, sunitinib, gemcitabine, and bleomycin than patients with a low CRGs score subtype [155]. Therefore, it is conceivable that the combinative treatment of copper compounds and chemotherapy drugs may have clinical benefits. This speculation was further exemplified in studies conducted by Hassan and coworkers. Their evidence suggested that 5-fluorouracil in combination with Cu and disulfiram presented a more markedly decreased proliferation of tumor cells and considerably ameliorated tumor burden with a remarkably decreased level of damage to cellular structures such as lipids, proteins, and DNA [156]. Likewise, Wang et al. found that disulfiram combined with 5-fluorouracil showed a remarkably antineoplastic activity with reduced metastatic and recurrence risk [154]. In the same vein, such results have also been consistently verified in other tumor models, such as colorectal cancer, pancreatic cancer, etc [157]. Clinical studies have reported significant elevated copper after radiotherapy in tumor patients, suggesting that serum copper levels may provide partial evidence for the efficacy of radiation therapy [158]. Copper treatment downregulated the expression of copper metabolism MURR1 domain 10 in animal models endowing cancer cells with increased resistance to radiation. Tetravinylpentylamine, a copper chelator, significantly decreased the serum level of copper and resensitized the tumor cells to radiation in mice fed copper, indicating that Tetravinylpentylamine is a copper-dependent selective radiosensitizer [159]. At the same time, targeting COMMD10 and related signaling may provide novel directions for discovering potential biomarkers and therapeutic strategies to alleviate and overcome radioresistance.

Combination with other agents. Both cuproptosis and ferroptosis are novel types of programmed necrosis. Whether there exists a connection between the two has aroused the curiosity of many researchers. Wang et al. explored the potential interaction between ferroptosis and cuproptosis in HCC. They found that two ferroptosis inducers, sorafenib and erastin, inhibited mitochondrial matrix-associated protease-mediated degradation

of FDX1, causing protein lipoylation and subsequent cuproptosis in hepatocellular carcinoma cells. Greater degrees of cell death could be observed when ferroptosis inducers and cuproptosis inducers were used simultaneously [160]. This discovery provides new insights into our further understanding of the role of cuproptosis and ferroptosis in the pathophysiological process and potential therapeutic targets of HCC.

Scores, models, and biomarkers

Based on the CRGs, the cuproptosis signature, [101] cuproptosis-related risk score, [10, 111] cuproptosis-related gene prognostic index have been developed to predict the prognosis and the TME of HCC [112]. The risk scores are positively correlated with M0 and M2 macrophages, while negatively correlated with $CD4^+$ T cells, $CD8^+$ T cells, and NK cells, indicating the suppressed immune TME [127]. The higher the CRG score, the worse the effect of immunotherapy [161]. Thus, those CRG scores can help judge the immune infiltration and the effect of immunotherapy. Furthermore, since CRGs are closely related to ICGs, targeting these CRGs can reverse the suppressive immune microenvironment, and improve the efficacy of ICIs and prognosis in HCC treatment [121].

Furthermore, various long non-coding RNAs (lncRNAs) can be used as biomarkers in HCC treatment (Table 3). The selected lncRNAs are associated with the prognosis of HCC, [162] the TME conditions, and expressions of key immune checkpoints [163]. Besides, the cuproptosis-related lncRNA signature, [164] the cuproptosis-related lncRNAs risk-scoring model, [162] the lncRNA profile [165] were built to predict the immune cell activity and prognosis of immunotherapy in HCC, as well as the targeted therapy evaluation.

FDX1 may have promise as a biomarker and therapeutic target based on preliminary studies, which is closely related to cancer mutation, immunity, and prognosis, [166] and is expected to become a new therapeutic target for HCC. FDX1 is down-expressed in various cancer cells, [166] including HCC, and loss of FDX1 renders cancer cells resistant to cuproptosis [16]. Clinical studies have shown that HCC patients with high FDX1 expression exhibited longer survival times [10]. Besides, FDX1 is highly associated with and directly targeted by elesclomol [167]. DLAT can also serve as a new predictive biomarker for HCC prognosis and is closely related to the TME and immune system of HCC patients [119]. HCC patients with reduced DLAT expression have a better prognosis with better OS and disease-specific survival [119]. Elesclomol is an anticancer drug that heavily relies on its transport of extracellular copper [96]. Through elesclomol administration, the FDX1 is activated, thereby inducing cuproptosis. Both preclinical and clinical trials have confirmed the safety and the cytotoxicity of elesclomol to treat cancer, [168–170] which deserves special attention in HCC therapy.

Challenges targeting cuproplasia and cuproptosis in HCC

Although copper compounds, nano-copper, and combination therapies seem to be poised to become part of standard medical practice, certain clinical trials have not yet released their findings, leading to uncertainties and inquiries regarding the efficacy of this approach to treating cancer [NCT00006332]. The lack of detailed experimental information hinders our ability to accurately

Table 3 The summary of research on cuproptosis-associated RNA biomarkers in HCC

Researchers	Biomarkers of RNA	Findings and Significance of the Research	References
Genhao Zhang et al	lncRNAs (AC099329.2, AC138904.1, AC145343.1, DNMBP-AS1, DEPDC1-AS1, GIHCG)	Selected six lncRNA linked to cuproptosis and built a lncRNA profile to predict the prognosis of immunotherapy in HCC	165
Qiongyue Zhang et al	lncRNAs (AC003093.1, AC015819.1, AL122035.1, AL590705.3, and MKLN1-AS)	Selected five lncRNAs that could be used in HCC immunotherapy and targeted therapy evaluation, immune cell activity, and function prediction	171
Hongfei Zhu et al	lncRNAs (AC005479.2, AC009974.2, GSEC, AC026412.3, AC245060.7, AL031985.3, AL158166.1, AL365361.1, LINC00426)	Built the CRlncSig to predict the prognosis of immunotherapy in HCC	164
Shujia Chen et al	lncRNAs (AC019069.1, AC079209.1, AC105020.5, HCG15, LINC01515)	Those lncRNAs are associated with TME and expressions of key immune checkpoints	163
Lan Luo et al	lncRNAs (AC012073.1, AC099850.3, AL031985.3, KDM4A-AS1, MIR210HG, MKLN1-AS, and PLBD1AS1)	Built the CRLRSM, and the high expression of these 7 lncRNAs is associated with poor prognosis in HCC	162
Ze Jin et al	miRNAs	The first research focused on miRNAs of cuproptosis in HCC and the CRMs associated with the prognosis of HCC	172

CRlncSig cuproptosis-related lncRNA signature, *CRLRSM* cuproptosis-related lncRNAs risk-scoring model, *CRMs* cuproptosis-related miRNAs, *HCC* hepatocellular carcinoma, *lncRNA* long non-coding RNA

determine the reasons for potential failures. Challenges such as inadequate clinical efficacy, flawed experimental design and methodologies, safety concerns regarding toxicity and side effects, poor drug-like properties, and limited commercial viability, collectively cast doubt on the future prospects of these treatments. The robust stability and prevalent presence of lncRNAs, as indicated in Table 3, suggest their potential utility as dependable cancer biomarkers. However, some non-coding RNAs lack specificity, as elevated levels may not always indicate a specific cancer type but could potentially be attributed to other malignancies or inflammatory responses triggered by bacterial and viral infections. Furthermore, discrepancies in findings across various studies investigating the same non-coding RNA may stem from factors such as sample size, statistical approaches, specificity of detection techniques, and other methodological considerations. Given the above shortcomings, non-coding RNA still faces great challenges in entering the clinical translation stage. Establishing standard operating procedures for evaluating non-coding RNA, including sample selection, RNA extraction, detection, and standardization methods, would facilitate the clinical application of non-coding RNA. Researchers have combined genomics, proteomics, metabolomics, etc. with clinical big data, artificial intelligence, and machine learning to develop various risk scores and prognostic models. These models help doctors make better clinical decisions about HCC and improve patient prognosis. However, due to the complexity and diversity in the pathogenesis, clinical behaviors, biology, pathology, and molecular characteristics of HCC subtypes, as well as variations in chemotherapeutic sensitivities and prognosis, the ability of prediction models established for a single subtype is subject to certain limitations, so that these models still face the dilemma of being difficult for further generalization. Building more comprehensive, more sensitive, and algorithmically better models remains a key challenge. Establishing closer connections between these models and immune infiltration may provide some inspiration for clinical treatment. Furthermore, there is an urgent need for a better understanding of the cross-talk between cuproplasia, cuproptosis, and other forms of cell death, such as ferroptosis. This insight might reveal a potential correlation not only for related cell and animal experiments assessing the candidate contribution of crosstalk between different types of cell death to certain diseases, but, more importantly, also in the coming time clinical research evaluating the efficacy of potential drugs attempting to provide more practical guidance for the combination therapy and clinical decision-making of diseases linked with cuproplasia and cuproptosis (e.g., cancer, neurodegenerative diseases, and obesity, as well as Wilson's disease).

Concluding remarks

HCC presents a significant global health challenge, exerting considerable strain on healthcare systems worldwide. An in-depth analysis of cuproplasia and cuproptosis mechanisms within the TME of HCC unveils their opposing nature and their impact on copper regulation within the TME. Cuproplasia fosters TME formation, angiogenesis, and metastasis, whereas cuproptosis may alleviate mitochondrial dysfunction and hypoxic conditions in the TME. Therefore, inhibiting cuproplasia and enhancing cuproptosis in HCC are essential for achieving therapeutic efficacy in HCC. These areas necessitate further investigation and offer promising research prospects.

Abbreviations

AMPK	Adenosine 5'-monophosphate(AMP)-activated protein kinase
ATOX	Antioxidant protein 1
ATP7B	Adenosine 5'-triphosphatase copper transporting beta
CAFs	Cancer-associated fibroblasts
COMMD	Copper metabolism MURR1 domain
COX	Cytochrome c oxidase
CRGs	Cuproptosis-related genes
DLAT	Dihydrolipoamide S-acetyltransferase
DSPK	Dual-specificity protein kinase
ECM	Extracellular matrix
FDX1	Ferredoxin 1
GSH	Glutathione
GSK3β	Glycogen synthase kinase-3β
HCC	Hepatocellular carcinoma
HMGB1	High-mobility group box 1
MDSCs	Myeloid-derived suppressor cells
MAPK	Mitogen-activated protein kinase
LOX	Lysyl oxidase
PARP1	Poly (ADP-ribose) polymerase 1
PD-L1	Programmed death-ligand 1
ROS	Reactive oxygen species
SLC31A1	Solute carrier family 31 member 1
TCA	Tricarboxylic acid
TME	Tumor microenvironment
TNF	Tumor necrosis factor
ULK	Unc-51 Like Autophagy Activating Kinase
VEGF	Vascular endothelial growth factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12935-025-03683-4.

Additional file 1.

Acknowledgements

Not applicable

Author contributions

Ruoyu Zhang and YunFei Tan: the original draft preparation, tables, and figures production, review, and revision, with the help of Ke Xu and Ning Huang; Liu Mei and Liming Wang: conceptualization, review, and critical revision; Jian Wang: conceptualization and English modification; Ruoyu Zhang and YunFei Tan should be considered co-first authors, Liu Mei and Liming Wang should be considered as co-corresponding authors; All authors: literature search, review, commentary, and final approval of the final version of the manuscript, including the authorship list.

Funding
Liming Wang is supported by the CAMS Innovation Fund for Medical Sciences (CIFMS), 2022-I2M-C&T-B-062.

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 19 May 2024 Accepted: 8 February 2025
Published online: 09 April 2025

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA A Cancer J Clinicians. 2022;72(1):7–33. https://doi.org/10.3322/caac.21708.
2. Hepatocellular carcinoma. *Nature Reviews Disease Primers.* 2021;7(1):7. https://doi.org/10.1038/s41572-021-00245-6
3. Lu C, Rong D, Zhang B, et al. Current perspectives on the immunosuppressive tumor microenvironment in hepatocellular carcinoma: challenges and opportunities. Mol Cancer. 2019;18(1):130. https://doi.org/10.1186/s12943-019-1047-6.
4. Oura K, Morishita A, Tani J, Masaki T. Tumor immune microenvironment and immunosuppressive therapy in hepatocellular carcinoma: a review. Int J Mol Sci. 2021;22(11):5801.
5. Zhao P, Malik S, Xing S. Epigenetic mechanisms involved in HCV-induced hepatocellular carcinoma (HCC). Front Oncol. 2021. https://doi.org/10.3389/fonc.2021.677926.
6. Zhu Q, Ma Y, Liang J, et al. AHR mediates the aflatoxin B1 toxicity associated with hepatocellular carcinoma. Signal Transduct Target Ther. 2021;6(1):299. https://doi.org/10.1038/s41392-021-00713-1.
7. Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol. 2019;16(7):411–28. https://doi.org/10.1038/s41575-019-0145-7.
8. Vogel A, Meyer T, Sapisochin G, Salem R, Saborowski A. Hepatocellular carcinoma. Lancet. 2022;400(10360):1345–62. https://doi.org/10.1016/s0140-6736(22)01200-4.
9. Ge EJ, Bush AI, Casini A, et al. Connecting copper and cancer: from transition metal signalling to metalloplasia. Nat Rev Cancer. 2022;22(2):102–13. https://doi.org/10.1038/s41568-021-00417-2.
10. Zhang Z, Zeng X, Wu Y, Liu Y, Zhang X, Song Z. Cuproptosis-related risk score predicts prognosis and characterizes the tumor microenvironment in hepatocellular carcinoma. Front Immunol. 2022. https://doi.org/10.3389/fimmu.2022.925618.
11. Blockhuys S, Celauro E, Hildesjö C, et al. Defining the human copper proteome and analysis of its expression variation in cancers. Metallomics. 2017;9(2):112–23. https://doi.org/10.1039/c6mt00202a.
12. Ding P, Zhang X, Jin S, et al. CD147 functions as the signaling receptor for extracellular divalent copper in hepatocellular carcinoma cells. Oncotarget. 2017;8(31):51151–63. https://doi.org/10.18632/oncotarget.17712.
13. Maung MT, Carlson A, Olea-Flores M, et al. The molecular and cellular basis of copper dysregulation and its relationship with human pathologies. Faseb J. 2021. https://doi.org/10.1096/fj.202100273RR.
14. Duan W-J, He R-R. Cuproptosis: copper-induced regulated cell death. Sci China Life Sci. 2022;65(8):1680–2. https://doi.org/10.1007/s11427-022-2106-6.
15. Kahlson MA, Dixon SJ. Copper-induced cell death. Science. 2022;375(6586):1231–2. https://doi.org/10.1126/science.abo3959.
16. Tsvetkov P, Coy S, Petrova B, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. Science. 2022;375(6586):1254–61. https://doi.org/10.1126/science.abf0529.
17. Balter V, Nogueira da Costa A, Bondanese VP, et al. Natural variations of copper and sulfur stable isotopes in blood of hepatocellular carcinoma patients. Proc Natl Acad Sci USA. 2015;112(4):982–5. https://doi.org/10.1073/pnas.1415151112.
18. Liu H, Guo H, Jian Z, et al. Copper induces oxidative stress and apoptosis in the mouse liver. Oxid Med Cell Longev. 2020;2020:1359164. https://doi.org/10.1155/2020/1359164.
19. Wu Z, Lv G, Xing F, et al. Copper in hepatocellular carcinoma: a double-edged sword with therapeutic potentials. Cancer Lett. 2023;571:216348. https://doi.org/10.1016/j.canlet.2023.216348.
20. Chen W, Yang A, Jia J, Popov YV, Schuppan D, You H. Lysyl oxidase (LOX) family members: rationale and their potential as therapeutic targets for liver fibrosis. Hepatology. 2020;72(2):729–41. https://doi.org/10.1002/hep.31236.
21. Zhan XH, Jiao JW, Zhang HF, et al. LOXL2 upregulates phosphorylation of ezrin to promote cytoskeletal reorganization and tumor cell invasion. Cancer Res. 2019;79(19):4951–64. https://doi.org/10.1158/0008-5472.Can-19-0860.
22. Tsang T, Posimo JM, Gudiel AA, Cicchini M, Feldser DM, Brady DC. Copper is an essential regulator of the autophagic kinases ULK1/2 to drive lung adenocarcinoma. Nat Cell Biol. 2020;22(4):412–24. https://doi.org/10.1038/s41556-020-0481-4.
23. Xu E, Chen M, Zheng J, Maimaitiming Z, Zhong T, Chen H. Deletion of hephaestin and ceruloplasmin induces a serious systemic iron deficiency and disrupts iron homeostasis. Biochem Biophys Res Commun. 2018;503(3):1905–10. https://doi.org/10.1016/j.bbrc.2018.07.134.
24. Xiao T, Ackerman CM, Carroll EC, et al. Copper regulates rest-activity cycles through the locus coeruleus-norepinephrine system. Nat Chem Biol. 2018;14(7):655–63. https://doi.org/10.1038/s41589-018-0062-z.
25. Salmi M, Jalkanen S. VAP-1: an adhesin and an enzyme. Trends Immunol. 2001;22(4):211–6. https://doi.org/10.1016/s1471-4906(01)01870-1.
26. Hordyjewska A, Popiołek Ł, Kocot J. The many "faces" of copper in medicine and treatment. Biometals. 2014;27(4):611–21. https://doi.org/10.1007/s10534-014-9736-5.
27. da Silva DA, De Luca A, Squitti R, et al. Copper in tumors and the use of copper-based compounds in cancer treatment. J Inorg Biochem. 2022;226:111634. https://doi.org/10.1016/j.jinorgbio.2021.111634.
28. Voli F, Valli E, Lerra L, et al. Intratumoral copper modulates PD-L1 expression and influences tumor immune evasion. Cancer Res. 2020;80(19):4129–44. https://doi.org/10.1158/0008-5472.Can-20-0471.
29. Bisaglia M, Bubacco L. Copper ions and Parkinson's disease: why is homeostasis so relevant? Biomolecules. 2020. https://doi.org/10.3390/biom10020195.
30. Lowe J, Taveira-da-Silva R, Hilário-Souza E. Dissecting copper homeostasis in diabetes mellitus. IUBMB Life. 2017;69(4):255–62. https://doi.org/10.1002/iub.1614.
31. Fukai T, Ushio-Fukai M, Kaplan JH. Copper transporters and copper chaperones: roles in cardiovascular physiology and disease. Am J Physiol Cell Physiol. 2018;315(2):186–201. https://doi.org/10.1152/ajpcell.00132.2018.
32. Oliveri V. Selective targeting of cancer cells by copper ionophores: an overview. Front Mol Biosci. 2022;9:841814. https://doi.org/10.3389/fmolb.2022.841814.
33. Davis CI, Gu X, Kiefer RM, Ralle M, Gade TP, Brady DC. Altered copper homeostasis underlies sensitivity of hepatocellular carcinoma to copper chelation. Metallomics. 2020;12(12):1995–2008. https://doi.org/10.1039/d0mt00156b.
34. Porcu C, Antonucci L, Barbaro B, et al. Copper/MYC/CTR1 interplay: a dangerous relationship in hepatocellular carcinoma. Oncotarget. 2018;9(10):9325–43. https://doi.org/10.18632/oncotarget.24282.
35. Dongiovanni P, Lanti C, Riso P, Valenti L. Nutritional therapy for nonalcoholic fatty liver disease. J Nutr Biochem. 2016;29:1–11. https://doi.org/10.1016/j.jnutbio.2015.08.024.

36. Liu X, Sun B, Yao Y, et al. Identification of copper metabolism and cuproptosis-related subtypes for predicting prognosis tumor microenvironment and drug candidates in hepatocellular carcinoma. Front Immunol. 2022;13:996308. https://doi.org/10.3389/fimmu.2022.996308.
37. Ginès P, Krag A, Abraldes JG, Solà E, Fabrellas N, Kamath PS. Liver cirrhosis. Lancet. 2021;398(10308):1359–76. https://doi.org/10.1016/s0140-6736(21)01374-x.
38. Zhang B, Li Y, Song L, et al. Cuproplasia characterization in colon cancer assists to predict prognosis and immunotherapeutic response. Front Oncol. 2023;13:1061084. https://doi.org/10.3389/fonc.2023.1061084.
39. Wang X, Zhou M, Liu Y, Si Z. Cope with copper: from copper linked mechanisms to copper-based clinical cancer therapies. Cancer Lett. 2023;561:216157. https://doi.org/10.1016/j.canlet.2023.216157.
40. Prasad S, Gupta SC, Tyagi AK. Reactive oxygen species (ROS) and cancer: role of antioxidative nutraceuticals. Cancer Lett. 2017;387:95–105. https://doi.org/10.1016/j.canlet.2016.03.042.
41. Shanbhag V, Jasmer-McDonald K, Zhu S, et al. ATP7A delivers copper to the lysyl oxidase family of enzymes and promotes tumorigenesis and metastasis. Proc Natl Acad Sci U S A. 2019;116(14):6836–41. https://doi.org/10.1073/pnas.1817473116.
42. Guan D, Zhao L, Shi X, Ma X, Chen Z. Copper in cancer: from pathogenesis to therapy. Biomed Pharmacother. 2023;163:114791. https://doi.org/10.1016/j.biopha.2023.114791.
43. Ruiz LM, Libedinsky A, Elorza AA. Role of copper on mitochondrial function and metabolism. Front Mol Biosci. 2021;8:711227. https://doi.org/10.3389/fmolb.2021.711227.
44. Keswani T, Mitra S, Bhattacharyya A. Copper-induced immunotoxicity involves cell cycle arrest and cell death in the liver. Environ Toxicol. 2015;30(4):411–21. https://doi.org/10.1002/tox.21916.
45. Garber K. Cancer's copper connections. Science. 2015;349(6244):129. https://doi.org/10.1126/science.349.6244.129.
46. Kitchin KT, Richards JA, Robinette BL, et al. Biochemical effects of copper nanomaterials in human hepatocellular carcinoma (HepG2) cells. Cell Biol Toxicol. 2022. https://doi.org/10.1007/s10565-022-09720-6doi:
47. Siddiqui MA, Alhadlaq HA, Ahmad J, Al-Khedhairy AA, Musarrat J, Ahamed M. Copper oxide nanoparticles induced mitochondria mediated apoptosis in human hepatocarcinoma cells. PLoS ONE. 2013;8(8):e69534. https://doi.org/10.1371/journal.pone.0069534.
48. Brady DC, Crowe MS, Turski ML, et al. Copper is required for oncogenic BRAF signalling and tumorigenesis. Nature. 2014;509(7501):492–6. https://doi.org/10.1038/nature13180.
49. Guo J, Cheng J, Zheng N, et al. Copper promotes tumorigenesis by activating the PDK1-AKT oncogenic pathway in a copper transporter 1 dependent Manner. Adv Sci (Weinh). 2021;8(18):e2004303. https://doi.org/10.1002/advs.202004303.
50. Dankner M, Rose AAN, Rajkumar S, Siegel PM, Watson IR. Classifying BRAF alterations in cancer: new rational therapeutic strategies for actionable mutations. Oncogene. 2018;37(24):3183–99. https://doi.org/10.1038/s41388-018-0171-x.
51. Sammons S, Brady D, Vahdat L, Salama AK. Copper suppression as cancer therapy: the rationale for copper chelating agents in BRAF(V600) mutated melanoma. Melanoma Manag. 2016;3(3):207–16. https://doi.org/10.2217/mmt-2015-0005.
52. Wachsmann J, Peng F. Molecular imaging and therapy targeting copper metabolism in hepatocellular carcinoma. World J Gastroenterol. 2016;22(1):221–31. https://doi.org/10.3748/wjg.v22.i1.221.
53. Fang AP, Chen PY, Wang XY, et al. Serum copper and zinc levels at diagnosis and hepatocellular carcinoma survival in the Guangdong Liver Cancer Cohort. Int J Cancer. 2019;144(11):2823–32. https://doi.org/10.1002/ijc.31991.
54. Krishnamoorthy L, Cotruvo JA Jr, Chan J, et al. Copper regulates cyclic-AMP-dependent lipolysis. Nat Chem Biol. 2016;12(8):586–92. https://doi.org/10.1038/nchembio.2098.
55. Liu X, Liang Y, Song R, et al. Long non-coding RNA NEAT1-modulated abnormal lipolysis via ATGL drives hepatocellular carcinoma proliferation. Mol Cancer. 2018;17(1):90. https://doi.org/10.1186/s12943-018-0838-5.
56. Cheng W, Cheng Z, Zhang C, Weng L, Xing D, Zhang M. Investigating the association between COMMD3 expression and the prognosis of hepatocellular carcinoma. J Cancer. 2022;13(6):1871–81. https://doi.org/10.7150/jca.62454.
57. Zheng L, You N, Huang X, et al. COMMD7 regulates NF-κB signaling pathway in hepatocellular carcinoma stem-like cells. Mol Ther Oncolytics. 2019;12:112–23. https://doi.org/10.1016/j.omto.2018.12.006.
58. Zhu T, Peng X, Cheng Z, et al. COMMD3 expression affects angiogenesis through the HIF1α/VEGF/NF-κB signaling pathway in hepatocellular carcinoma In Vitro and In Vivo. Oxid Med Cell Longev. 2022;2022:1655502. https://doi.org/10.1155/2022/1655502.
59. Lawal G, Xiao Y, Rahnemai-Azar AA, et al. The immunology of hepatocellular carcinoma. Vaccines (Basel). 2021. https://doi.org/10.3390/vaccines9101184.
60. Wu M, Huang Q, Xie Y, et al. Improvement of the anticancer efficacy of PD-1/PD-L1 blockade via combination therapy and PD-L1 regulation. J Hematol Oncol. 2022;15(1):24. https://doi.org/10.1186/s13045-022-01242-2.
61. Mo Z, Liu D, Rong D, Zhang S. Hypoxic characteristic in the immunosuppressive microenvironment of hepatocellular carcinoma. Front Immunol. 2021;12:611058. https://doi.org/10.3389/fimmu.2021.611058.
62. Yang M, Li J, Gu P, Fan X. The application of nanoparticles in cancer immunotherapy: targeting tumor microenvironment. Bioact Mater. 2021;6(7):1973–87. https://doi.org/10.1016/j.bioactmat.2020.12.010.
63. Chen C, Wang Z, Ding Y, Qin Y. Tumor microenvironment-mediated immune evasion in hepatocellular carcinoma. Front Immunol. 2023;14:1133308. https://doi.org/10.3389/fimmu.2023.1133308.
64. Liu Y, Cao X. Characteristics and significance of the pre-metastatic niche. Cancer Cell. 2016;30(5):668–81. https://doi.org/10.1016/j.ccell.2016.09.011.
65. Horwitz E, Stein I, Andreozzi M, et al. Human and mouse VEGFA-amplified hepatocellular carcinomas are highly sensitive to sorafenib treatment. Cancer Discov. 2014;4(6):730–43. https://doi.org/10.1158/2159-8290.Cd-13-0782.
66. Rostamian H, Khakpoor-Koosheh M, Jafarzadeh L, et al. Restricting tumor lactic acid metabolism using dichloroacetate improves T cell functions. BMC Cancer. 2022;22(1):39. https://doi.org/10.1186/s12885-021-09151-2.
67. Kciuk M, Kołat D, Kałuzińska-Kołat Ż, et al. PD-1/PD-L1 and DNA damage response in cancer. Cells. 2023. https://doi.org/10.3390/cells12040530.
68. Zhou B, Guo L, Zhang B, et al. Disulfiram combined with copper induces immunosuppression via PD-L1 stabilization in hepatocellular carcinoma. Am J Cancer Res. 2019;9(11):2442–55.
69. He X, Xu C. Immune checkpoint signaling and cancer immunotherapy. Cell Res. 2020;30(8):660–9. https://doi.org/10.1038/s41422-020-0343-4.
70. Ribas A. Adaptive immune resistance: how cancer protects from immune attack. Cancer Discov. 2015;5(9):915–9.
71. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. Nat Immunol. 2015;16(4):343–53. https://doi.org/10.1038/ni.3123.
72. Chaintreuil P, Kerreneur E, Bourgoin M, et al. The generation, activation, and polarization of monocyte-derived macrophages in human malignancies. Front Immunol. 2023;14:1178337. https://doi.org/10.3389/fimmu.2023.1178337.
73. Zhao HJ, Zhao XH. Modulatory effect of the supplemented copper ion on in vitro activity of bovine lactoferrin to murine splenocytes and RAW264.7 macrophages. Biol Trace Elem Res. 2019;189(2):519–28. https://doi.org/10.1007/s12011-018-1472-1.
74. Tang X, Yan Z, Miao Y, et al. Copper in cancer: from limiting nutrient to therapeutic target. Front Oncol. 2023;13:1209156. https://doi.org/10.3389/fonc.2023.1209156.
75. Peng H, Zhu E, Zhang Y. Advances of cancer-associated fibroblasts in liver cancer. Biomark Res. 2022;10(1):59. https://doi.org/10.1186/s40364-022-00406-z.
76. Pei L, Liu Y, Liu L, et al. Roles of cancer-associated fibroblasts (CAFs) in anti- PD-1/PD-L1 immunotherapy for solid cancers. Mol Cancer. 2023;22(1):29. https://doi.org/10.1186/s12943-023-01731-z.
77. Yin Z, Dong C, Jiang K, et al. Heterogeneity of cancer-associated fibroblasts and roles in the progression, prognosis, and therapy of hepatocellular carcinoma. J Hematol Oncol. 2019;12(1):101. https://doi.org/10.1186/s13045-019-0782-x.
78. Craig AJ, von Felden J, Garcia-Lezana T, Sarcognato S, Villanueva A. Tumour evolution in hepatocellular carcinoma. Nat Rev

Gastroenterol Hepatol. 2020;17(3):139–52. https://doi.org/10.1038/s41575-019-0229-4.
79. Paul S, Lal G. The molecular mechanism of natural killer cells function and its importance in cancer immunotherapy. Front Immunol. 2017;8:1124. https://doi.org/10.3389/fimmu.2017.01124.
80. Cheng F, Peng G, Lu Y, et al. Relationship between copper and immunity: the potential role of copper in tumor immunity. Front Oncol. 2022;12:1019153. https://doi.org/10.3389/fonc.2022.1019153.
81. Chang W, Li H, Zhong L, et al. Development of a copper metabolism-related gene signature in lung adenocarcinoma. Front Immunol. 2022;13:1040668. https://doi.org/10.3389/fimmu.2022.1040668.
82. Wang X, He S, Zheng X, et al. Transcriptional analysis of the expression, prognostic value and immune infiltration activities of the COMMD protein family in hepatocellular carcinoma. BMC Cance. 2021;21(1):1001. https://doi.org/10.1186/s12885-021-08699-3.
83. Fang W, Gan Y, Zhang L, Xiong J. COMMD2 upregulation mediated by an ncRNA Axis correlates with an unfavorable prognosis and tumor immune infiltration in liver hepatocellular carcinoma. Front Oncol. 2022;12:853026. https://doi.org/10.3389/fonc.2022.853026.
84. Lin HY, Li CJ, Yang YL, Huang YH, Hsiau YT, Chu PY. Roles of Lysyl oxidase family members in the tumor microenvironment and progression of liver cancer. Int J Mol Sci. 2020. https://doi.org/10.3390/ijms21249751.
85. Li R, Wang Y, Zhang X, et al. Exosome-mediated secretion of LOXL4 promotes hepatocellular carcinoma cell invasion and metastasis. Mol Cancer. 2019;18(1):18. https://doi.org/10.1186/s12943-019-0948-8.
86. Radić J, Kožik B, Nikolić I, et al. Multiple roles of LOXL2 in the progression of hepatocellular carcinoma and its potential for therapeutic targeting. Int J Mol Sci. 2023. https://doi.org/10.3390/ijms241411745.
87. Xing X, Wang Y, Zhang X, et al. Matrix stiffness-mediated effects on macrophages polarization and their LOXL2 expression. Febs J. 2021;288(11):3465–77. https://doi.org/10.1111/febs.15566.
88. Tan HY, Wang N, Zhang C, Chan YT, Yuen MF, Feng Y. Lysyl Oxidase-Like 4 Fosters an Immunosuppressive Microenvironment During Hepatocarcinogenesis. Hepatology. 2021;73(6):2326–41. https://doi.org/10.1002/hep.31600.
89. Yi M, Zheng X, Niu M, Zhu S, Ge H, Wu K. Combination strategies with PD-1/PD-L1 blockade: current advances and future directions. Mol Cancer. 2022;21(1):28. https://doi.org/10.1186/s12943-021-01489-2.
90. Budimir N, Thomas GD, Dolina JS, Salek-Ardakani S. Reversing T-cell exhaustion in cancer: lessons learned from PD-1/PD-L1 immune checkpoint blockade. Cancer Immunol Res. 2022;10(2):146–53. https://doi.org/10.1158/2326-6066.Cir-21-0515.
91. Li Y. Copper homeostasis: emerging target for cancer treatment. IUBMB Life. 2020;72(9):1900–8. https://doi.org/10.1002/iub.2341.
92. Yang M, Liu J, Wang F, et al. Lysyl oxidase assists tumor-initiating cells to enhance angiogenesis in hepatocellular carcinoma. Int J Oncol. 2019;54(4):1398–408. https://doi.org/10.3892/ijo.2019.4705.
93. Yang Z, Yang S, Misner BJ, Liu-Smith F, Meyskens FL. The role of APE/Ref-1 signaling pathway in hepatocellular carcinoma progression. Int J Oncol. 2014;45(5):1820–8. https://doi.org/10.3892/ijo.2014.2589.
94. Lutsenko S. Dynamic and cell-specific transport networks for intracellular copper ions. J Cell Sci. 2021. https://doi.org/10.1242/jcs.240523.
95. Liu H. Pan-cancer profiles of the cuproptosis gene set. Am J Cancer Res. 2022;12(8):4074–81.
96. Zheng P, Zhou C, Lu L, Liu B, Ding Y. Elesclomol: a copper ionophore targeting mitochondrial metabolism for cancer therapy. J Exp Clin Cancer Res. 2022;41(1):271. https://doi.org/10.1186/s13046-022-02485-0.
97. Li SR, Bu LL, Cai L. Cuproptosis: lipoylated TCA cycle proteins-mediated novel cell death pathway. Signal Transduct Target Ther. 2022;7(1):158. https://doi.org/10.1038/s41392-022-01014-x.
98. Tang D, Chen X, Kroemer G. Cuproptosis: a copper-triggered modality of mitochondrial cell death. Cell Res. 2022;32(5):417–8. https://doi.org/10.1038/s41422-022-00653-7.
99. Liu J, Liu Y, Wang Y, Kang R, Tang D. HMGB1 is a mediator of cuproptosis-related sterile inflammation. Front Cell Dev Biol. 2022;10:996307. https://doi.org/10.3389/fcell.2022.996307.
100. Kang R, Tang D, Schapiro NE, et al. The HMGB1/RAGE inflammatory pathway promotes pancreatic tumor growth by regulating mitochondrial bioenergetics. Oncogene. 2014;33(5):567–77. https://doi.org/10.1038/onc.2012.631.
101. Chen X, Hu G, Xiong L, Xu Q. Relationships of cuproptosis-related genes with clinical outcomes and the tumour immune microenvironment in hepatocellular carcinoma. Pathol Oncol Res. 2022;28:1610558. https://doi.org/10.3389/pore.2022.1610558.
102. Sun B, Ding P, Song Y, et al. FDX1 downregulation activates mitophagy and the PI3K/AKT signaling pathway to promote hepatocellular carcinoma progression by inducing ROS production. Redox Biol. 2024;75:103302. https://doi.org/10.1016/j.redox.2024.103302.
103. Ke C, Dai S, Xu F, et al. Cuproptosis regulatory genes greatly contribute to clinical assessments of hepatocellular carcinoma. BMC Cancer. 2023;23(1):25. https://doi.org/10.1186/s12885-022-10461-2.
104. Hidalgo F, Ferretti AC, Etichetti CB, et al. Alpha lipoic acid diminishes migration and invasion in hepatocellular carcinoma cells through an AMPK-p53 axis. Sci Rep. 2024;14(1):21275. https://doi.org/10.1038/s41598-024-72309-y.
105. Ye Y, Yu B, Wang H, Yi F. Glutamine metabolic reprogramming in hepatocellular carcinoma. Front Mol Biosci. 2023;10:1242059. https://doi.org/10.3389/fmolb.2023.1242059.
106. Song L, Zeng R, Yang K, Liu W, Xu Z, Kang F. The biological significance of cuproptosis-key gene MTF1 in pan-cancer and its inhibitory effects on ROS-mediated cell death of liver hepatocellular carcinoma. Discov Oncol. 2023;14(1):113. https://doi.org/10.1007/s12672-023-00738-8.
107. Zhou Y, Wang XB, Qiu XP, Shuai Z, Wang C, Zheng F. CDKN2A promoter methylation and hepatocellular carcinoma risk: A meta-analysis. Clin Res Hepatol Gastroenterol. 2018;42(6):529–41. https://doi.org/10.1016/j.clinre.2017.07.003.
108. Li Y, Yu Z. Pan-cancer analysis reveals copper transporters as promising potential targets. Heliyon. 2024;10(17):e37007. https://doi.org/10.1016/j.heliyon.2024.e37007.
109. Herzig S, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol. 2018;19(2):121–35. https://doi.org/10.1038/nrm.2017.95.
110. Wang Y, Zhang Y, Wang L, et al. Development and experimental verification of a prognosis model for cuproptosis-related subtypes in HCC. Hepatol Int. 2022;16(6):1435–47. https://doi.org/10.1007/s12072-022-10381-0.
111. Fu J, Wang S, Li Z, et al. Comprehensive multiomics analysis of cuproptosis-related gene characteristics in hepatocellular carcinoma. Front Genet. 2022;13:942387. https://doi.org/10.3389/fgene.2022.942387.
112. Ding L, Li W, Tu J, et al. Identification of cuproptosis-related subtypes, cuproptosis-related gene prognostic index in hepatocellular carcinoma. Front Immunol. 2022. https://doi.org/10.3389/fimmu.2022.989156.
113. Liu Z, Qi Y, Wang H, Zhang Q, Wu Z, Wu W. Risk model of hepatocellular carcinoma based on cuproptosis-related genes. Front Genet. 2022;13:1000652. https://doi.org/10.3389/fgene.2022.1000652.
114. Cong T, Luo Y, Liu Y, et al. Cuproptosis-related immune checkpoint gene signature: prediction of prognosis and immune response for hepatocellular carcinoma. Front Genet. 2022;13:1000997. https://doi.org/10.3389/fgene.2022.1000997.
115. Li J, Li B, Zhao R, Li G. Systematic analysis of the aberrances and functional implications of cuproptosis in cancer. Iscience. 2023;26(4):106319. https://doi.org/10.1016/j.isci.2023.106319.
116. Xiao J, Liu Z, Wang J, Zhang S, Zhang Y. Identification of cuprotosis-mediated subtypes, the development of a prognosis model, and influence immune microenvironment in hepatocellular carcinoma. Front Oncol. 2022;12:941211. https://doi.org/10.3389/fonc.2022.941211.
117. Boutilier AJ, Elsawa SF. Macrophage polarization states in the tumor microenvironment. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22136995.
118. Zhou SL, Zhou ZJ, Hu ZQ, et al. Tumor-associated neutrophils recruit macrophages and t-regulatory cells to promote progression of hepatocellular carcinoma and resistance to sorafenib. Gastroenterology. 2016;150(7):1646-1658.e1617. https://doi.org/10.1053/j.gastro.2016.02.040.
119. Zhou Y, Gu H, Shao B, et al. Glycolysis-related gene dihydrolipoamide acetyltransferase promotes poor prognosis in hepatocellular carcinoma through the Wnt/β-catenin and PI3K/Akt signaling pathways. Ann Transl Med. 2022;10(22):1240. https://doi.org/10.21037/atm-22-5272.
120. Tong X, Tang R, Xiao M, et al. Targeting cell death pathways for cancer therapy: recent developments in necroptosis, pyroptosis, ferroptosis,

and cuproptosis research. J Hematol Oncol. 2022;15(1):174. https://doi.org/10.1186/s13045-022-01392-3.
121. Li Y, Zou L, Liu X, Luo J, Liu H. Identification of immune-related genes for establishment of prognostic index in hepatocellular carcinoma. Front Cell Dev Biol. 2021;9:760079. https://doi.org/10.3389/fcell.2021.760079.
122. Zhou Y, Lin F, Wan T, et al. ZEB1 enhances Warburg effect to facilitate tumorigenesis and metastasis of HCC by transcriptionally activating PFKM. Theranostics. 2021;11(12):5926–38. https://doi.org/10.7150/thno.56490.
123. Icard P, Shulman S, Farhat D, Steyaert JM, Alifano M, Lincet H. How the Warburg effect supports aggressiveness and drug resistance of cancer cells? Drug Resist Updat. 2018;38:1–11. https://doi.org/10.1016/j.drup.2018.03.001.
124. Zhang D, Li Z, Li T, et al. miR-517a promotes Warburg effect in HCC by directly targeting FBP1. Onco Targets Ther. 2018;11:8025–32. https://doi.org/10.2147/ott.S172084.
125. Gao F, Zhang X, Wang S, et al. TSP50 promotes the Warburg effect and hepatocyte proliferation via regulating PKM2 acetylation. Cell Death Dis. 2021;12(6):517. https://doi.org/10.1038/s41419-021-03782-w.
126. Zuo Q, He J, Zhang S, et al. PPARγ coactivator-1α suppresses metastasis of hepatocellular carcinoma by inhibiting warburg effect by PPARγ-Dependent WNT/β-catenin/pyruvate dehydrogenase kinase isozyme 1 axis. Hepatology. 2021;73(2):644–60. https://doi.org/10.1002/hep.31280.
127. Zhao X, Chen J, Yin S, et al. The expression of cuproptosis-related genes in hepatocellular carcinoma and their relationships with prognosis. Front Oncol. 2022;12:992468. https://doi.org/10.3389/fonc.2022.992468.
128. Yang T, Zhang Y, Chen J, Sun L. Crosstalk between autophagy and immune cell infiltration in the tumor microenvironment. Front Med (Lausanne). 2023;10:1125692. https://doi.org/10.3389/fmed.2023.1125692.
129. Jiang GM, Tan Y, Wang H, et al. The relationship between autophagy and the immune system and its applications for tumor immunotherapy. Mol Cancer. 2019;18(1):17. https://doi.org/10.1186/s12943-019-0944-z.
130. Xia H, Green DR, Zou W. Autophagy in tumour immunity and therapy. Nat Rev Cancer. 2021;21(5):281–97. https://doi.org/10.1038/s41568-021-00344-2.
131. Wen H, Qu C, Wang Z, et al. Cuproptosis enhances docetaxel chemosensitivity by inhibiting autophagy via the DLAT/mTOR pathway in prostate cancer. Faseb J. 2023;37(9):e23145. https://doi.org/10.1096/fj.202300980R.
132. Aubert L, Nandagopal N, Steinhart Z, et al. Copper bioavailability is a KRAS-specific vulnerability in colorectal cancer. Nat Commun. 2020;11(1):3701. https://doi.org/10.1038/s41467-020-17549-y.
133. Thai SF, Jones CP, Robinette BL, et al. Effects of copper nanoparticles on mRNA and small RNA expression in human hepatocellular carcinoma (HepG2) Cells. J Nanosci Nanotechnol. 2021;21(10):5083–98. https://doi.org/10.1166/jnn.2021.19328.
134. Wang J, Luo C, Shan C, et al. Inhibition of human copper trafficking by a small molecule significantly attenuates cancer cell proliferation. Nat Chem. 2015;7(12):968–79. https://doi.org/10.1038/nchem.2381.
135. Stehling O, Lill R. The role of mitochondria in cellular iron-sulfur protein biogenesis: mechanisms, connected processes, and diseases. Cold Spring Harb Perspect Biol. 2013. https://doi.org/10.1101/cshperspect.a011312.
136. Steinbrueck A, Sedgwick AC, Brewster JT 2nd, et al. Transition metal chelators, pro-chelators, and ionophores as small molecule cancer chemotherapeutic agents. Chem Soc Rev. 2020;49(12):3726–47. https://doi.org/10.1039/c9cs00373h.
137. Zhang W, Chen C, Shi H, et al. Curcumin is a biologically active copper chelator with antitumor activity. Phytomedicine. 2016;23(1):1–8. https://doi.org/10.1016/j.phymed.2015.11.005.
138. Yoshii J, Yoshiji H, Kuriyama S, et al. The copper-chelating agent, trientine, suppresses tumor development and angiogenesis in the murine hepatocellular carcinoma cells. Int J Cancer. 2001;94(6):768–73. https://doi.org/10.1002/ijc.1537.
139. Sone K, Maeda M, Wakabayashi K, et al. Inhibition of hereditary hepatitis and liver tumor development in Long-Evans cinnamon rats by the copper-chelating agent trientine dihydrochloride. Hepatology. 1996;23(4):764–70. https://doi.org/10.1053/jhep.1996.v23.pm0008666330.
140. Oliveri V, Vecchio G. Prochelator strategies for site-selective activation of metal chelators. J Inorg Biochem. 2016;162:31–43. https://doi.org/10.1016/j.jinorgbio.2016.05.012.
141. Bakthavatsalam S, Sleeper ML, Dharani A, George DJ, Zhang T, Franz KJ. leveraging γ-Glutamyl transferase to direct cytotoxicity of copper dithiocarbamates against prostate cancer cells. Angew Chem Int Ed Engl. 2018;57(39):12780–4. https://doi.org/10.1002/anie.201807582.
142. Gao F, Yuan Y, Ding Y, Li PY, Chang Y, He XX. DLAT as a cuproptosis promoter and a molecular target of elesclomol in hepatocellular carcinoma. Curr Med Sci. 2023;43(3):526–38. https://doi.org/10.1007/s11596-023-2755-0.
143. Huang L, Wu F, Wang Q, et al. TME-triggered copper-coordinated engineered programmable nanogenerators for on-demand cascade-amplifying oxidative stress. J Mater Chem B. 2023;11(16):3679–92. https://doi.org/10.1039/d3tb00032j.
144. Chen Z, Wu Y, Yao Z, et al. 2D Copper(II) metalated metal-organic framework nanocomplexes for dual-enhanced photodynamic therapy and amplified antitumor immunity. ACS Appl Mater Interfaces. 2022;14(39):44199–210. https://doi.org/10.1021/acsami.2c12990.
145. Xia Q, Zhang Y, Zhang H, et al. Copper nanocrystalline-doped folic acid-based super carbon dots for an enhanced antitumor effect in response to tumor microenvironment stimuli. J Mater Chem B. 2022;10(39):8046–57. https://doi.org/10.1039/d2tb01363k.
146. Zhang K, Meng X, Yang Z, Dong H, Zhang X. Enhanced cancer therapy by hypoxia-responsive copper metal-organic frameworks nanosystem. Biomaterials. 2020;258:120278. https://doi.org/10.1016/j.biomaterials.2020.120278.
147. Ning S, Mo J, Huang R, et al. Injectable thermo-sensitive hydrogel loaded hollow copper sulfide nanoparticles for ROS burst in TME and effective tumor treatment. Front Bioeng Biotechnol. 2023;11:1191014. https://doi.org/10.3389/fbioe.2023.1191014.
148. Li T, Rao B, Xu D, et al. Enzyme-like copper-encapsulating magnetic nanoassemblies for switchable T1-weighted MRI and potentiating chemo-/photo-dynamic therapy. Acta Biomater. 2022;153:431–41. https://doi.org/10.1016/j.actbio.2022.09.062.
149. Ding F, Li F, Tang D, et al. Restoration of the immunogenicity of tumor cells for enhanced cancer therapy via nanoparticle-mediated copper chaperone inhibition. Angew Chem Int Ed Engl. 2022;61(31):e202203546. https://doi.org/10.1002/anie.202203546.
150. Xu Q, Li Q, Yang Z, et al. Lenvatinib and Cu(2–x)S nanocrystals co-encapsulated in poly(D, L-lactide-co-glycolide) for synergistic chemo-photothermal therapy against advanced hepatocellular carcinoma. J Mater Chem B. 2021;9(48):9908–22. https://doi.org/10.1039/d1tb01808f.
151. Nan L, Yuan W, Guodong C, Yonghui H. Multitargeting strategy using tetrathiomolybdate and lenvatinib: maximizing antiangiogenesis activity in a preclinical liver cancer model. Anticancer Agents Med Chem. 2023;23(7):786–93. https://doi.org/10.2174/1871520622666220907115027.
152. Cheng AL, Kang YK, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009;10(1):25–34. https://doi.org/10.1016/s1470-2045(08)70285-7.
153. Wang J, Li J, Liu J, et al. Interplay of ferroptosis and cuproptosis in cancer: dissecting metal-driven mechanisms for therapeutic potentials. Cancers (Basel). 2024. https://doi.org/10.3390/cancers16030512.
154. Wang Z, Tan J, McConville C, et al. Poly lactic-co-glycolic acid controlled delivery of disulfiram to target liver cancer stem-like cells. Nanomedicine. 2017;13(2):641–57. https://doi.org/10.1016/j.nano.2016.08.001.
155. Li Y, Zeng X. A novel cuproptosis-related prognostic gene signature and validation of differential expression in hepatocellular carcinoma. Front Pharmacol. 2022;13:1081952. https://doi.org/10.3389/fphar.2022.1081952.
156. Hassan I, Ebaid H, Alhazza IM, Al-Tamimi J, Rady AM. Disulfiram enhances the antineoplastic activity and sensitivity of murine hepatocellular carcinoma to 5-FU via redox management. Pharmaceuticals (Basel). 2023. https://doi.org/10.3390/ph16020169.
157. Hendrych M, Říhová K, Adamová B, et al. Disulfiram increases the efficacy of 5-fluorouracil in organotypic cultures of colorectal carcinoma.

Biomed Pharmacother. 2022;153:113465. https://doi.org/10.1016/j.biopha.2022.113465.
158. Hsu HY, Lin SY, Huang CJ, Lian SL, Ho YH. Changes of serum copper and zinc levels in patients with nasopharyngeal carcinoma by radiotherapy. Biol Trace Elem Res. 1994;46(1–2):1–13. https://doi.org/10.1007/bf02790064.
159. Yang M, Wu X, Hu J, et al. COMMD10 inhibits HIF1α/CP loop to enhance ferroptosis and radiosensitivity by disrupting Cu-Fe balance in hepatocellular carcinoma. J Hepatol. 2022;76(5):1138–50. https://doi.org/10.1016/j.jhep.2022.01.009.
160. Wang W, Lu K, Jiang X, et al. Ferroptosis inducers enhanced cuproptosis induced by copper ionophores in primary liver cancer. J Exp Clin Cancer Res. 2023;42(1):142. https://doi.org/10.1186/s13046-023-02720-2.
161. Zhao S, Chen S, Liu W, et al. Integrated machine learning and bioinformatic analyses used to construct a copper-induced cell death-related classifier for prognosis and immunotherapeutic response of hepatocellular carcinoma patients. Front Pharmacol. 2023;14:1188725. https://doi.org/10.3389/fphar.2023.1188725.
162. Luo L, Hu X, Huang A, et al. A noval established cuproptosis-associated LncRNA signature for prognosis prediction in primary hepatic carcinoma. Evidence-Based Complem Alternative Med. 2022;2022:2075638. https://doi.org/10.1155/2022/2075638.
163. Chen S, Liu P, Zhao L, et al. A novel cuproptosis-related prognostic lncRNA signature for predicting immune and drug therapy response in hepatocellular carcinoma. Front Immunol. 2022;13:954653. https://doi.org/10.3389/fimmu.2022.954653.
164. Zhu H, Mao F, Wang K, Feng J, Cheng S. Cuproptosis-related lncRNAs predict the clinical outcome and immune characteristics of hepatocellular carcinoma. Front Genet. 2022;13:972212. https://doi.org/10.3389/fgene.2022.972212.
165. Zhang G, Sun J, Zhang X. A novel Cuproptosis-related LncRNA signature to predict prognosis in hepatocellular carcinoma. Sci Rep. 2022;12(1):11325. https://doi.org/10.1038/s41598-022-15251-1.
166. Zhang C, Zeng Y, Guo X, et al. Pan-cancer analyses confirmed the cuproptosis-related gene FDX1 as an immunotherapy predictor and prognostic biomarker. Front Genet. 2022;13:923737. https://doi.org/10.3389/fgene.2022.923737.
167. Tsvetkov P, Detappe A, Cai K, et al. Mitochondrial metabolism promotes adaptation to proteotoxic stress. Nat Chem Biol. 2019;15(7):681–9. https://doi.org/10.1038/s41589-019-0291-9.
168. Modica-Napolitano JS, Bharath LP, Hanlon AJ, Hurley LD. The anticancer agent elesclomol has direct effects on mitochondrial bioenergetic function in isolated mammalian mitochondria. Biomolecules. 2019. https://doi.org/10.3390/biom9080298.
169. Nagai M, Vo NH, Shin Ogawa L, et al. The oncology drug elesclomol selectively transports copper to the mitochondria to induce oxidative stress in cancer cells. Free Radic Biol Med. 2012;52(10):2142–50. https://doi.org/10.1016/j.freeradbiomed.2012.03.017.
170. Hedley D, Shamas-Din A, Chow S, et al. A phase I study of elesclomol sodium in patients with acute myeloid leukemia. Leuk Lymphoma. 2016;57(10):2437–40. https://doi.org/10.3109/10428194.2016.1138293.
171. Zhang Q, Huang Y, Xia Y, Liu Y, Gan J. Cuproptosis-related lncRNAs predict the prognosis and immune response in hepatocellular carcinoma. Clin Exp Med. 2022. https://doi.org/10.1007/s10238-022-00892-3.
172. Jin Z, Wang M, Meng Y, et al. Prognostic implication of a cuproptosis-related miRNA signature in hepatocellular carcinoma. J Health Eng. 2022;2022:4694323. https://doi.org/10.1155/2022/4694323.
173. Khan S, Zafar A, Naseem I. Redox cycling of copper by coumarin-di(2-picolyl)amine hybrid molecule leads to ROS-mediated modulation of redox scavengers, DNA damage and cell death in diethylnitrosamine induced hepatocellular carcinoma. Bioorg Chem. 2020;99:103818. https://doi.org/10.1016/j.bioorg.2020.103818.
174. Li D, Shi Z, Liu X, et al. Identification and development of a novel risk model based on cuproptosis-associated RNA methylation regulators for predicting prognosis and characterizing immune status in hepatocellular carcinoma. Hepatol Int. 2023;17(1):112–30. https://doi.org/10.1007/s12072-022-10460-2.
175. Ji Y, Dai F, Zhou B. Designing salicylaldehyde isonicotinoyl hydrazones as Cu(II) ionophores with tunable chelation and release of copper for hitting redox Achilles heel of cancer cells. Free Radic Biol Med. 2018;129:215–26. https://doi.org/10.1016/j.freeradbiomed.2018.09.017.
176. Dong T, Jiang J, Zhang H, et al. PFP@PLGA/Cu(12)Sb(4)S(13)-mediated PTT ablates hepatocellular carcinoma by inhibiting the RAS/MAPK/MT-CO1 signaling pathway. Nano Converg. 2021;8(1):29. https://doi.org/10.1186/s40580-021-00279-2.
177. Piret JP, Jacques D, Audinot JN, et al. Copper(II) oxide nanoparticles penetrate into HepG2 cells, exert cytotoxicity via oxidative stress and induce pro-inflammatory response. Nanoscale. 2012;4(22):7168–84. https://doi.org/10.1039/c2nr31785k.
178. Yang Z, Zhao Z, Cheng H, Shen Y, Xie A, Zhu M. In-situ fabrication of novel Au nanoclusters-Cu(2+)@sodium alginate/hyaluronic acid nanohybrid gels for cuproptosis enhanced photothermal/photodynamic/chemodynamic therapy via tumor microenvironment regulation. J Colloid Interface Sci. 2023;641:215–28. https://doi.org/10.1016/j.jcis.2023.03.065.

Publisher's Note