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Advances in Cuproptosis of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) has already become a severe health risk and brings a lot of healthcare burden to the world. Apart from traditional HCC treatment strategies (surgery, liver transplantation, etc.), the emergence of immunotherapy targeting the immune microenvironment of HCC has brought new promise to patients with advanced HCC. However, adverse effects like drug resistance still exist. The liver is the main organ for storing copper ions, in copper overload can lead to liver function impairment and even the development of HCC. In recent years, a new mode of cell death has been identified, namely cuproptosis, a mode of programmed cell death that is dependent on copper ions and the tricarboxylic acid (TCA) cycle with mitochondria. Interestingly, a potential relationship between cuproptosis and the development of HCC has been found. Conclusively, this review provides an in-depth discussion of copper homeostasis in humans, the mechanism of cuproptosis, the potential impact of cuproptosis with HCC, and the therapeutic modalities of HCC that target cuproptosis, which provide new insights to promote the development of research targeting cuproptosis in HCC.

Keywords

Hepatocellular Carcinoma, Cuproptosis, Copper Homeostasis, Therapy

1. Introduction

Hepatocellular carcinoma (HCC), one of the most common malignant tumors, is lethal and has become a severe threat to the lives of people [1]. Copper is a common metallic element essential for all living things, including humans, to sustain life [2]. It is important to note that copper levels must be maintained

within a defined range to ensure normal biochemical processes, or it may lead to the development of certain diseases (e.g., Wilson's disease, HCC, etc.) [3]. Overloading of copper leads to the development of cuproptosis, a copper-dependent, non-apoptotic form of cell death, which may be associated with the development of several types of cancer [4]. The occurrence of cuproptosis is closely related to proteins such as ferredoxin 1 (FDX1) and dihydrolipoyl transacetylase (DLAT), as they are involved in direct interactions between copper ions and lipoylated components of the tricarboxylic acid (TCA) cycle, contributing to cell death [5]. Due to the relatively insidious onset of HCC, a large number of patients are already at an advanced stage by the time they are diagnosed, and the best time has been lost. However, current treatments for advanced HCC have limited effectiveness, and people have to search for new and more effective therapies to deal with HCC to improve the prognosis of patients with HCC [6]. A growing number of studies have found that cuproptosis is associated with the prognosis of HCC and that targeting cuproptosis is achievable for treating HCC [7] [8]. Therefore, this review will systematically review the association between HCC and cuproptosis, which will help us find new therapeutic targets for HCC targeting cuproptosis to improve the outcomes of HCC patients.

2. Hepatocellular Carcinoma

HCC is not only the 6th most common malignant tumor worldwide but also the 4th most significant contributor to the number of tumor-related deaths [9]. Although the prevalence and death rates of HCC are on the decline in traditional at-risk areas such as Japan and China, they are on the rise in Northern America and some European regions. HCC mainly results from chronic and persistent liver injury, with common risk factors including chronic hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infection, chronic excessive alcohol consumption, non-alcoholic fatty liver disease, and aflatoxin poisoning [10]. Surgical resection, liver transplantation, radiotherapy, and intra-arterial therapy, including transcatheter arterial chemoembolization (TACE), are now common treatments for HCC [1]. Of these, surgical tumor removal is the preferred treatment option for eradicating HCC in patients with HCC; however, the overwhelming majority of patients with HCC are in the intermediate to late stage of the disease when they are diagnosed, and the opportunity for surgical removal has already been lost. Although other treatments can somewhat improve the prognosis of HCC patients, it is still not promising [11]. The emergence of immunotherapies that target the immune system has brought new expectations to a wide range of HCC patients [12]. The immunotherapies for HCC mainly include immune checkpoint inhibitors (ICIs), cancer vaccines, T cell receptor (TCR)-T cell therapy, and chimeric antigen receptor (CAR)-T cell therapy [6] [13] [14]. Notably, immune checkpoint inhibitors have dramatically reshaped the management of HCC. The combination regimen of atezolizumab (an ICI targeting PD-L1) and bevacizumab (an anti-VEGF drug) is already used for first-line treatment of HCC. However, different immune classifications of HCC respond differently to

ICIs, which can be roughly divided into inflammatory (hot) and non-inflammatory (cold) HCC tumors, with the "cold" tumors being less effective in response to ICI therapy [15] [16]. Moreover, immune resistance remains a crucial limiting factor in developing ICIs [17]. With the discovery of the gut-liver axis, on the other hand, there has been a growing awareness of the significance of gut microbiota for the treatment of HCC, as they can directly or indirectly shape the tumor microenvironment (TME) to impact tumor progression. Targeting gut microbiota and its metabolites through antibiotics, probiotic agents, and fecal microbiota transplantation has emerged as a new promising therapeutic option for HCC [18]-[20]. Nevertheless, the mechanisms of the gut microbiota and its metabolites affecting HCC have not been thoroughly investigated, and the therapeutic effects of related treatments on HCC have not yet been clarified, so scientists still need to make further research on this subject in the future for the benefit of HCC patients. Recently, a new mode of programmed cell death, cuproptosis, has been discovered, and it has also been found that it has a potential association with a variety of cancers [4]. In the following, we will systematically introduce the mechanism underlying the onset of cuproptosis and the implications of cuproptosis for HCC.

3. Copper Homeostasis and Cuproptosis

3.1. Copper Homeostasis

Copper is one of the metallic trace elements required for the maintenance of normal life activities, and it can switch between the reduction state Cu(I) and the oxidation state Cu(II) [21]. Adults normally have about 100 mg of copper in their bodies and get their daily dose of copper, typically ~1.5 mg/day, through primarily dietary sources [22]. Due to its distinctive architecture, copper is an indispensable catalytic cofactor of redox reactions as well as an essential structural constituent of many enzymes, which mainly include ceruloplasmin (CP), cytochrome c oxidase (CCO), and Cu/Zn superoxide dismutase 1 (SOD1) [23]. Copper deficiency leads to reduced activity of several enzymes, resulting in abnormal glucose metabolism, dyslipidaemia and even suppression of the immune system. Conversely, when copper is in excess, it causes cellular damage by promoting oxidative stress, DNA damage, and inflammation [24]. The liver is the main organ responsible for regulating copper metabolism, which is critical for copper homeostasis in the body as copper is taken up from the food intake mainly through the small intestine and then transported to the liver via CP and albumin, which are the principal haemocarriers. Most of the copper liberated by the liver can be carried to various tissues (e.g., brain, etc.) through CP transportation or excreted from the body by biliary excretion. In contrast, surplus copper is preserved in the liver cells through the metallothionein MT1/MT2 [25] [26]. Copper homeostasis is of utmost significance to the cell, as excess copper ions have a non-negligible toxic effect on the cells. For example, copper binds, inhibits its enzymatic activity, and diverts electrons during reactions that consistently

produce harmful oxidation radicals [27].

Indeed, unbalanced copper homeostasis not only affects the respiration of mitochondria but also causes alterations in lipid metabolism, glycolysis, and insulin resistance and even leads to the onset of disease [2]. Copper ATPases (Cu - ATPases) are widespread membrane-spanning proteins belonging to the P-ATPases (P-type ATPases) that can utilize the power of ATP to partake in cellular copper transport between the cytoplasm and distinct organelles, as well as the expulsion of excess copper from the cell [28]. It's noteworthy that copper transporter ATPase A (ATP7A) and copper transporter ATPase B (ATP7B) are highly relevant to human copper homeostasis. ATP7A transports copper absorbed in the intestines to the portal vein, and subsequently, copper can be transferred to the liver through carriers such as CP and albumin. Meanwhile, ATP7B transports excess copper from the liver cells into the bile when the body is overloaded with copper, allowing it to be excreted through the bile ducts [23]. Deficiencies of ATP7A and ATP7B function can lead to disruption of copper homeostasis and can even cause the development of certain diseases. As an example, the loss of function of ATP7A leads to Menkes disease, a deadly inherited copper deficiency that happens in infants, causing gradual neural damage and even death [29]. Malfunction of ATP7B contributes to the development of Wilson's disease, an inherited copper metabolism deficiency disease, which leads to a slowdown in hepatic synthesis of CP, a marked decrease in biliary copper excretion, thus leading to copper deposition in organs such as the liver and the brain, with clinical symptoms of damage to the corresponding organs [30]. Interestingly, Cai *et al.* found that repair of the *ATP7B* gene in liver cells by reprogramming can significantly reduce excess copper accumulation with associated inflammation and fibrosis in mouse livers [31]. Separately, copper transport protein 1 (CTR1) is a member of the solute carrier family of copper permease (SLC31), which is one of the essential proteins mediating cellular copper uptake and also one of the essential proteins for maintaining copper homeostasis [32]. Negative feedback regulation of the cellular CTR1-copper axis is an effective way to maintain intracellular copper homeostasis. Concretely, when extracellular copper levels are increased, CTR1 will be endocytosed to eliminate copper cell toxicity, while CTR1 will be recirculated back to the cell membrane when extracellular copper levels are decreased [33].

3.2. Cuproptosis and Its Mechanism of Occurrence

Tsvetkov's team recently discovered a new approach to cell death known as cuproptosis, a cell death that relies on mitochondrial respiration. They found that, differently from other modes of cell death (e.g., ferroptosis), cuproptosis takes place through direct binding of copper to the lipoylated elements (such as DLAT) of the TCA cycle [34]. Briefly, mitochondria are the primary damaging organelle in cellular cuproptosis, resulting in oxidative impairment of the mitochondrial membrane and enzymatic functional compromise of the TCA cycle [3]. When a cell undergoes cuproptosis, it will show the following morphological

changes: breakage of the cell membrane, wrinkling of mitochondria, impairment of the endoplasmic reticulum, disruption of chromatin, and so on [35]. The *FDX1* gene is thought to be an influential regulator of cuproptosis, encoding a reducing enzyme for converting Cu(II) to the greater cytotoxicity of Cu(I) [36]. Elesclomol, a high-efficiency copper ion carrier, can promote cuproptosis by suppressing the function of the FDX1 protein, which is a crucial protein in the biosynthesis of iron-sulfur (Fe-S) clusters. At the same time, as a reducing substrate for FDX1, the elesclomol-Cu(II) complex causes the production of Cu(I) and reactive oxygen species (ROS), further promoting cuproptosis [37]. In addition, FDX1 regulates protein lipoylation directly by combining with the lipoyl synthase (LIAS) enzyme and facilitating its functional integration with the lipoyl carrier protein glycine cleavage system protein H (GCSH), which is functionally significant for the mitochondrial TCA cycle [38]. Xiong *et al.* speculated that p53 may be possibly associated with cuproptosis onset, mediates the glycolytic to oxidative phosphorylation switch, and potentiates the biogenesis of Fe-S clusters [36]. Interestingly, cuproptosis has some potential associations with other cellular death modalities. For example, elesclomol causes iron death in colorectal cancer cells by increasing Cu(II) levels in mitochondria and decreasing the expression of the Cu(II) transporter ATP7A, resulting in the build-up of ROS, thus promoting the degradation of SLC7A11 [39]. During cuproptosis, acute mitochondrial impairment and toxic stress can lead to the activation of caspases, proteases that are involved in the execution of cell death. Necrosis pathways may activate, leading to inflammation, membrane rupture, and cell swelling [40].

In summary, copper homeostasis is crucial for maintaining normal life activities in the human body. Briefly, the regulation of copper homeostasis is a complex and involved process by several biological proteins, including CTR1, ATP7A/ATP7B, MT1/MT2, and others (see **Table 1**). Copper overload leads to the development of cuproptosis, which has a non-negligible role in the development of certain diseases, and we will detail the potential relationship and mechanisms of cuproptosis and HCC below.

Table 1. Functions of copper homeostasis and cuproptosis-related factors.

Factor	Abbreviation	Function description	References
Ferredoxin 1	FDX1	Convert Cu(II) to the greater cytotoxicity of Cu(I)	[36]
Lipoyl synthase	LIAS	Catalyze the conversion of octanoylated domains into lipoylated derivatives	[38]
Dihydrolipoyl transacetylase	DLAT	Involve indirect interactions between copper ions and lipoylated components of the TCA cycle	[5]
Metallothionein 1	MT1	Preserve excess copper in liver cells	[25] [26]

Continued

Metallothionein 2	MT2	Preserve excess copper in liver cells	[25] [26]
Copper transport protein 1	CTR1	Mediate cellular copper uptake	[32] [33]
Copper transporter ATPase A	ATP7A	Transport copper absorbed in the intestines to the portal vein	[23]
Copper transporter ATPase B	ATP7B	Transport excess copper from the liver cells into the bile when the body is overloaded with copper	[23]
Ceruloplasmin	CP	Transport carrier for copper	[25]
Iron-sulfur clusters	Fe-S clusters	An essential carrier of electrons	[37]

4. Effects of Cuproptosis on Hepatocellular Carcinoma

A growing number of studies have identified a potential relationship between the development of HCC and cuproptosis. Copper levels have been demonstrated to be strongly associated with HCC, and serum copper and CP levels may be considered markers for HCC detection [41]. Moreover, disturbed copper homeostasis is usually observable in HCC patients, and the serum Copper level is associated positively with BCLC stage and alanine aminotransferase (ALT) [42]. Increased levels of reductioactive free copper in the liver can catalyze the Fenton reaction and generate high levels of hydroxyl radicals in rat models of Wilson's disease, which may contribute to acute hepatitis and even HCC [43]. The increased incidence of HCC in patients and animal models with Wilson disease suggests that copper overload may contribute to the development of HCC through unknown mechanisms [44]. Similarly, the study confirmed that tumors may require higher levels of copper than healthy tissue [45]. Earlier research showed that HCC patients have higher serum ceruloplasmin levels than cirrhotic patients [46]. Excessive accumulation of copper in the liver induces HIF-1α activation, which in turn causes hepatocellular carcinoma. Concretely, serum HIF-1α levels in HCC patients correlated remarkably with serum copper levels, and copper overload led to the up-regulation of HIF-1α expression, which would have promoted the genes responsible for angiogenesis to be transcribed in HCC patients [47]. The expression of ATP7A was high in HCC tissues and positively correlated with the expression of DLAT in HCC. Knockdown of ATP7A increased copper accumulation in HCC cells, decreased DLAT expression, and suppressed HCC cell proliferation. Notably, ATP7A was positively correlated not only with the expression of immune checkpoints such as PD-1 and CTLA 4, but also closely associated with various immune cells such as $CD4^+$ T cells, $CD8^+$ T cells, and NK cells [48].

Maternal embryonic leucine zipper kinase (MELK) promotes the expression of the cuprotoosis-related signature gene *DLAT* through the PI3K/mTOR pathway. It reshapes mitochondrial functionality, contributing to elesclomol resistance and, eventually, HCC progress [48]. Six Transmembrane Epithelial An-

tigen of Prostate 2 (STEAP2) is a member of the metalloreductase family that indirectly assists in the intake of copper and iron ions. Knocking down the expression of STEAP2 in HCC cells can effectively reduce intracellular copper content and suppress the c-Jun N-terminal kinase (JNK) and p38 phosphorylation, thereby inhibiting the development of HCC [49]. *ARID1A*, one of the frequently muted tumor suppressive genes in HCC, has been shown to suppress the expression of *PKM* (a vital gene involved in glycolysis), and its deficiency induces glucose metabolism to be reprogrammed, meaning that cells become more dependent on the TCA cycle than on glycolysis. It is important to note that *ARID1A* deficiency renders HCC cells more sensitive to cuproptosis [8]. *FDX1* is down-regulated in HCC, and its high expression is associated with adverse outcomes for HCC patients. Cuproptosis-related risk score (CRRS) reconstructions based on *FDX1* and its relevant genes indicated that HCC patients with high CRRS had a shorter duration of existence and higher tumor-immune cell infiltration [50]. Peng *et al.* clustered cuproptosis-related genes (CRGs) to derive the differentially expressed cuproptosis-related-genes (DECRGs), and found that *CFHR4*, *DNASE1L3*, *SPP2*, and *TAF6* are DECRGs for which high correlation can be found [51]. They also found that *TAF6* expression was remarkably greater in HCC cell lines than in normal hepatic cells and that high levels of *TAF6* expression in HCC patients correlated with a negative outcome. Rather, suppression of *TAF6* expression could repress the multiplication and migration of HCC cells and the expression of PD-1 proteins. In addition, Wang *et al.* predicted that the important CRGs affecting the prognosis of HCC are *COX17*, *SNCA*, and *PRNP*, which may affect the infiltration of immune cells in HCC and also correlate remarkably with the PD-L1, PD-1, and CTLA4 expressions [7]. Zhang's team identified 6 cuproptosis-associated differentially expressed lncRNAs (DE-lncRNAs) for prognostic prediction of HCC patients, that is, AC099329.2 and DNMBP-AS1 are protection lncRNAs, whereas AC138904.1, AC145343.1, GIHCG and DEPDC1-AS1 are non-protective lncRNAs [52].

Although many genes, lncRNAs, and others are currently predicted to be associated with cuproptosis, they have not yet been fully experimentally verified, and in the future, scientists will need to provide a large amount of experimental evidence to improve the theoretical system of cuproptosis. To sum up, cuproptosis is firmly associated with HCC, and the discovery of cuproptosis provides new perspectives for developing new effective therapies for HCC.

5. Targeting Cuproptosis for Hepatocellular Carcinoma Treatment

Eleschomol is an anti-cancer drug that targets cuproptosis by delivering copper ions to the mitochondria and causing an increase in ROS production, leading to tumor cells cuproptosis [53] [54]. Nevertheless, the treatment of HCC with eleschomol may have drawbacks, such as drug resistance [48]. Troxerutin with copper showed a notable anti-HCC effect, inducing HCC cell death by producing ROS without any poisonous effect on normal liver cells. Moreover, Troxeru-

tin therapy reduced the liver copper levels of rats with HCC [55]. Voli's study identified a strong correlation between CTR1 and programmed cell death ligand 1 (PD-L1) expression in tumor tissues. Supplemental copper-induced the expression of PD-L1 at both mRNA and protein levels in tumor cells, and RNA sequencing showed that copper modulates critical PD-L1-driven signaling pathways for immunological evasion in tumors. Contrarily, using copper chelators promoted PD-L1 degradation and enhanced infiltration of tumor-infiltrating $CD8^+$ T and natural killer (NK) cells, inhibited tumor growth, and increased mice survivability [56]. A finding that disulfiram combined with copper (DSF/Cu) remarkably induced HCC cellular cuproptosis by down-regulating FDX1 and Fe-S proteins and enhanced the sensitivity to cellular ferroptosis, resulting in an anti-HCC effect [57]. Moreover, the anti-HCC effect of DSF/Cu plus anti-PD-1 antibody co-treatment was significantly superior to that of single-agent treatment [58]. The expression of Cu and copper metabolism MURR1 domain 10 (COMMD10) of HCC showed less than normal hepatic tissues, and it inhibited cell multiplication and facilitated apoptosis through the inhibition of NF-κB signaling [59]. In addition, COMMD10 potentiates ferroptosis and sensitivity to radiotherapy through inhibition of the HIF-1*α*/CP loop to disrupt the Cu-Fe balance of HCC [60]. Curcumin, a polyphenol derived from turmeric, can treat HCC by inducing the expression of certain genes associated with cuproptosis and ferroptosis, such as *CYP1A1* and *MTTP* [61]. LINC02362 is a lncRNA that can regulate HCC progression. It was found that enhancement of the LINC02362/FDX1 axis suppressed HCC multiplication and increased the sensibility of HCC cells towards oxaliplatin via the cuproptosis mechanism [62]. The expression of brain-expressed X-linked protein 1 (BEX1) in HCC cells and tissues was higher than that in normal hepatocytes and tissues, and the deficiency of BEX1 inhibited the proliferation, invasion, and migration of HCC cells. In addition, BEX1 can combine with sorafenib in a stabilizing conformation, indicating that BEX1 could be a promising therapeutic target for mediating cuproptosis for HCC [63]. Based on deep learning, LGOd1 is considered to be a new anti-HCC drug with a characteristic levoglucosenone (LGO) scaffold, which may lead to cuproptosis in HCC cells by interacting with the copper chaperone for superoxide dismutase (CCS), thereby interfering with intracellular copper homeostasis [64].

Interestingly, copper-containing complexes can also exert distinct anti-HCC effects through different pathways. For instance, [Cu (ttpy-tpp)Br2]Br (CTB)is a Cu(II) complex targeted to mitochondria which can interfere with the functionality of HCC cell mitochondria to perform the anti-HCC property. Specifically, CTB can induce mitochondrial membrane potential collapsing to facilitate ROS acceleration and Dynamin-related protein 1 (Drp1) activation, causing apoptosis in HCC cells [65]. Combining lenvatinib on copper-containing nanomedicine carriers delivers lenvatinib efficiently and enhances the reactivity effectiveness of copper-based Fenton chemistry for HCC therapy [66]. Zhou *et al.* constructed a photothermal-triggered nanoplatform, Au@MSN-Cu/PEG/DSF, which enters

the tumor cells and releases DSF and Cu(II) to induce cellular cuproptosis [67]. Qiao's team developed copper-quinone-GOx nanoparticles (CQG NPs) that not only trigger cuproptosis in tumor cells by releasing copper ions, but also reshape the immune-suppressive TME to promote immune cell infiltration into the tumor [68]. For breast cancer, ZIF90/Cu-prodigiosin@PEG nanoplatform (ZCProP) induces cellular cuproptosis by targeting the delivery of copper and prodigiosin, which releases Cu(II) that promotes the aggregation of lipoylated proteins and the loss of Fe-S cluster proteins, as well as inducing mitochondrial dysfunction and DNA damage [69].

An increasing number of drugs targeting cuproptosis have been developed, especially those based on copper-based nanomaterials, which exhibit high biocompatibility and are able to enhance tumor therapeutic efficacy by triggering cuproptosis in tumor cells [70]. It could be argued that altering intracellular copper concentrations may be a prospective therapeutic strategy for HCC. However, the clinical efficacy of therapies associated with cuproptosis is limited by adverse effects or inadequate efficacy at tolerated doses [71]. Although therapeutic strategies for HCC targeting cuproptosis have shown new promise to patients with HCC, the relevant research is not yet sufficient, and more cellular, animal and even human experiments need to be investigated in the future for the development of more efficient and safer anti-HCC drugs.

6. Summary and Prospect

HCC is a malignant neoplastic disease with insidious onset and high lethality, which has already imposed a heavy healthcare burden on humanity. Because of the insidious onset of HCC, a lot of HCC patients are found to be in the advanced stage of cancer and miss the best time for treatment. Nowadays, the emergence of immunotherapies targeting the immune microenvironment of HCC, represented by ICIs, has brought new perspectives to patients with advanced HCC, and their emergence has improved the prognosis of HCC patients to a certain extent. However, they still have non-negligible drawbacks, such as drug resistance. Copper is a metallic trace element essential for the survival of mammals and is necessary for many normal physiological activities in the human body. Copper homeostasis is so important for maintaining human health that disruption of copper homeostasis is unfavorable to the human body. The liver is the main organ for reserving copper ions, and excess copper ions can lead to liver function damage, certain diseases (such as Menkes disease and Wilson's disease), and even the development of HCC. In recent years, a new mode of cell death, cuproptosis, has been identified as a programmed cell death dependent on copper ions and the mitochondrial tricarboxylic acid (TCA) cycle. Importantly, it has been found that targeting cuproptosis can be useful in achieving anti-HCC and improving patient prognosis. Although drugs targeting cuproptosis are showing new promise in the experimental phase, there is still a lack of understanding of cuproptosis, the underlying relationship between cuproptosis and

HCC has not yet been studied, and there are still many issues with related drugs. Currently, the lack of reliable biomarkers of cuproptosis and relevant randomized clinical trials to confirm a direct relationship between cuproptosis and HCC is limiting the use of cuproptosis-related drugs in the clinic. So, in the future, scientists should put more effort into research to improve the prognosis of HCC patients. Taken together, this review provides insights into the copper homeostasis in the human body, the mechanisms of cuproptosis, the potential impact of cuproptosis on HCC, and HCC treatments targeting cuproptosis, which offer new insights to facilitate the development of research targeting cuproptosis in HCC. Notably, the relevance of copper overload to HCC cell cuproptosis and how cuproptosis can be exploited for clinical tumor therapy needs further investigation.

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Conflicts of Interest

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