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Progress in the research of cuproptosis and possible targets for cancer therapy

cuproptosis and possible target for cancer therapy

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Abstract

Developing novel cancer therapies that exploit programmed cell death pathways holds great promise for advancing cancer treatment. According to a recently published study in Science, copper death (cuproptosis) occurs when intracellular copper is overloaded, triggering aggregation of lipidated mitochondrial proteins and Fe-S cluster proteins. This intriguing phenomenonis triggered by the instability of copper ions. Understanding the molecular mechanisms behind cuproptosis and its associated genes,as identified by Tsvetkov, including FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1, GLS, and CDKN2A, may open new avenues for cancer therapy. Here, we provide a new understanding of the role of copper death and related genes in cancer.

Key Words: cuproptosis; cuproptosis-related genes; cancer; targeted therapy

Core Tip: Cuproptosis-related genes are identified by Tsvetkov, including FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, PDHB, MTF1, GLS, and CDKN2A. Here, we provide a new understanding of the role of copper death and related genes in cancer.

INTRODUCTION

Tsvetkov *et al* have proposed an intriguing new form of programmed cell death related to the mitochondrial tricarboxylic acid (TCA) cycle, resulting in proteotoxic stress and copper-induced death, referred to as cuproptosis. These forms of oxidative stress-induced cell death are characterized by mitochondrial stress, including the accumulation of fatty acylated mitochondrial enzymes and the loss of Fe-S cluster proteins(1). The dysregulation of copper homeostasis promotes cancer growth and causes irreversible cellular damage. A variety of mechanisms have been suggested for copper's ability to induce cell death, such as oxidative stress, proteasome inhibition, and anti-angiogenesis(2).

The exact molecular mechanism underlying cuproptosis remains unclear, but recent studies have shed light on potential contributors. For instance, knockout of the FDX1 gene attenuates copper ionophore-induced cell death. Additionally, genes associated with the loss of lipidated mitochondrial enzymes and Fe-S cluster proteins loss, such as LIAS, LIPT1, and DLAT, among others, may contribute to cuproptosis(1, 3).

Although the precise correlation between cuproptosis and cancer is yet to be fully understood, imbalances in copper homeostasis have been implicated in cancer growth and cause irreversible cellular damage. Copper metabolism *in vivo* and cancer therapy has been extensively studied (4, 5). Certain genes involved in the cuproptosis pathway,such as FDX1, may also play a role in cancer development, serving as a key regulator of proptosis and associated with poor prognoses in specific cancer types (6). Here, we review the progress of copper ions in cancer therapy, the function of cuproptosis-related genes in cancer, and the possible target in cuproptosis.

COPPER IONS AND CANCER THERAPY

Recent studies have revealed three distinct mechanisms through which copper ions may induce cancer cell death: 1) Oxidative stress induction: Clinical anti-cancer drug ELESCOMOL has been found to exert its therapeutic effects through the transfer of copper ions to mitochondria, leading to oxidative stress (7). Furthermore, Liu *et al* (8)demonstrated that flavonoids can induce mitochondrial apoptosis through the modification of copper ions' redox cycle. 2) Inhibition of proteasomes: Chen *et al* (9) synthesized copper diethyldithiocarbamate (Cu(DDC)2) nanoparticles (NPs) that improved the resistance of prostate cancer to treatment. Copper ion-mediated endoplasmic reticulum (ER) stress is induced by proteasome inhibition and accumulation of ubiquitinated proteins. Proteasome inhibitors like bortezomib and carfilzomib have been explored for their potential as cancer treatment optionsin the form of various complexes,such as clioquinol and dithiocarbamates(10). 3) Reduce angiogenesis: Copper ions play a significant role in endothelial cell migration, proliferation, and fibronectin synthesis, crucial steps in angiogenesis(11, 12). However, Cu depletion can act as an anti-angiogenic switch, blocking the growth of endothelial cells and preventing their proliferation. By inhibiting copper transporters or chaperones like ATOX1 and CTR-1, in addition to direct capture of intracellular copper, copper imbalance can be induced, leading to anti-angiogenic effects (13, 14). Combining this approach with vascular targeting techniques, such as immunotherapy, can enhance the cancer-killing effects (15).The tumor micro environment (TME) is a complex ecosystem where various immune cells interact and influence tumor growth and progression(16, 17). In the early stage of tumor growth, neutrophils promote inflammation and tumor cell apoptosis by releasing cytokines. However, in the middle and late stages of tumor formation, neutrophils contribute to angiogenesis, accelerating tumor progression and local infiltration. Different T cell populations are involved in TME, among which CD8+T cells can target and destroy tumor cells, secrete interferon, and inhibit angiogenesis. CD4+T cells coordinate immune responses, with Th1 cells promoting cancer and Treg cells promoting tumor formation and survival, by secreting auxin and cytokines, which then interacts with fibroblasts and epithelial cells. Although less

prevalent than T cells,tumor-infiltrating B cells have anti-tumor effects,including antigen presentation to T cells, production of anti-tumor antibodies, and secretion of cytokines that promote cytotoxic immune responses. Regulatory B cells, on the other hand, promote tumors by producing cytokines that promote the immunosuppressive phenotype in macrophages, neutrophils, and Cytotoxic T cells. Tumor-associated macrophages (TAMs) are the predominant immune cells in the tumor micro environment. They are involved in coordinating cancer-related inflammation and can release CSF-1 to recruit TAMs, which have been implicated in cancer development. Moreover, TAMs can release EGF, modify cancer cells, and accelerate cell migration and metastasis. Medullary suppressive cells promote tumor invasion by weakening innate and adaptive anti-tumor responses.

In light of the mechanisms described above for copper ions in cancer treatment, copper complexes have been extensively studied for their potential in anti-cancer therapy. For instance, copper-amino acid sulfhydryl nanoparticles (Cu-CysNPs) can reduce Cu2+ to Cu+ when reacting with localized GSH. The generated Cu+ then reacts with hydrogen peroxide, resulting in an increase in ROS levels. Excessive ROS can induce apoptosis of cancer cells (18). A copper-containing complex known as Cu-TSC is another widely used complex to enhance TSC's cytotoxicity and ROS production(19). Chronic inflammation in the body can induce carcinogenesis and facilitate cancer spread. Copper complexes containing non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat inflammation and prevent cancer development. In breast cancer stem cell (CSC)-like cells, Boodram *et al* (20) demonstrated that Cu-NSAID complexes could induce ROS accumulation, DNA damage, and COX-2 inhibition. Furthermore, copper complexes with subcellular targeting properties can deliver more precise attacks on cancer cells. Kaur *et al* (21)reported that copper complexes containing polypyridine ligands could enter the endoplasmic reticulum *in situ*, leading to increased ROS levels and ER stress-induced immunogenic cell death in cancer cells (22). While copper complex-related therapies hold promise as a new anticancer strategy, their biocompatibility and application safety are critical challenges. Researchers have shown

that copper complexes are cancer-killing, but long-term stability and biosafety tests remain to be conducted before these therapies can be translated into clinical applications.

THE ROLE OF CUPROPTOSIS-RELATED GENES IN CANCER

Cuproptosis remains an area of active exploration in its relationship with cancer. However, significant research has been conducted to understand the mechanisms through which cuproptosis-related gene molecules contribute to cancer development (Table 2). Figure 2 illustrates how these genes induce cuproptosis.

FDX1

FDX1 is a ferredoxin protein primarily found in mitochondria, with diverse physiological functions, including the conversion of cytochromes during steroid hormone synthesis and vitamin D metabolism(23). Shi *et al* demonstrated that FDX1 is critical for Fe-S cluster biogenesis(24). Recent research has identifiedFDX1 as a key gene in the regulation of cuproptosis(25). Zhang's study found that FDX1 expression did not significantly differ across clinical stages in most cancers (26). Although the reduction in FDX1 expression may not directly impact the growth, apoptosis, or cell cycle distribution of LUAD cells, it could affect theirmetabolism,as FDX1 knockout has been shown to promote glycolysis and fatty acid oxidation. Further investigations into the mechanisms of FDX1 in cancer pathogenesis revealed significant positive correlations between FDX1 expression and immune cells in most cancers. FDX1 has been associated with MHC, immune activation, immune suppression, chemokines, and chemotaxis(27). Additionally, the products of factor receptors were co-positively expressed with FDX1, except for ACC and THCA. This indicates that FDX1 expression is closely related to the immune response of cancer cells, which has implications for prognosis and represents a potential target for immunosuppressants(28, 29). Given the crucial role of copper ions in cuproptosis, FDX1's significance as a key gene in this process makes it an intriguing target for cancer therapy. Studies exploring its role may offer valuable insights as it

directly influences the protein fatty acylation cycle, leading to the aggregation of these proteins and interference with respiratory chain iron-sulfur cluster proteins.

LIAS

LIAS encodes a protein belonging to the biotin and lipoic acid synthase families. Located in the mitochondria, this iron-sulfur enzyme contributes to lipoic acid biosynthesis,serving as the final step in the process. Diseases like diabetes, atherosclerosis, and neonatal epilepsy are associated with a lack of LIAS expression. Current studies on the association between the LIAS gene and cancer have predominantly focused on lung cancer(29).

Using *in situ* hybridization (ISH) and real-time quantitative PCR (qPCR), Dlamini *et al* investigated the differential expression of the LIAS gene in normal lung tissue and lung cancer samples. Their findings suggest that alteration in LIAS expression levels can promote lung cancer development, making LIAS an attractive target for novel therapies (29). However further studies are warranted to confirm its therapeutic effectiveness.

LIPT1

As a member of the fatty acyltransferase family, LIPT1 encodes an enzyme that catalyzes the transfer of fatty acyl groups from fatty acyl-AMPs to specific lysine residues in fatty acids-dependent enzymes. LIPT1-related disorders include fatty acyltransferase 1 deficiency and leukodystrophy(30). While there have been relatively few studies on LIPT1 in cancer, Chen conducted a systematic investigation of genes related to prognosis in bladder cancer using the pathological atlas of The Cancer Genome Atlas. Their findings revealed a correlation betweenLIPT1 expression and bladder cancer prognosis(31). However, further research is needed to elucidate the role of LIPT1 in other cancer types.

DLD

Diahydrolipid amide dehydrogenase, encoded by the DLD gene, is an essential enzyme that significantly impacts cell metabolism, particularly pyruvate metabolism and the tricarboxylic acid cycle(32). There is evidence that DLD could be used as a cancer-targeted therapy. In head and neck squamous cell carcinoma, DLD has been shown to be closely related to cystine deprivation and glutaminolysis.DLD's biological function enhances mitochondrial KDH, MMP, and glutaminase activity. Increasing mitochondrial iron levels can facilitate mitochondrial lipid peroxidation, or silencing DLD,which effectively reduces the proportion of cells undergoing death from cystine deprivation and reduces ROS levels in cystine-deprived cells. These processes have been closely related to cancer-programmed death(33). Patients with endometrial cancer have exhibited abnormal levels of immunoglobulin (Ig)A and non-DLD IgG auto antibodies in their sera, indicating a correlation with mitochondrial dihydrolipoamide dehydrogenase (DLD) protein(34). Moreover, comparing DLD protein expression levels between breast cancer and normal tissues revealed significant differences,highlighting the potential of DLD as a diagnostic and therapeutic target in breast cancer (35). Using DLDH-based exogenous ROS to target skin cancer cells, Avraham *et al* developed a method for targeting cancer cells, which could be a potential approach for melanoma treatment in the future(36).

DLAT

Dihydrolipoamide transacetylase (DLAT) is an essential component of the pyruvate dehydrogenase complex,along with dihydrolipoamide dehydrogenase and pyruvate dehydrogenase. This enzyme complex plays a crucial role in the synthesis of pyruvate acetyl-CoA. As the sole enzyme capable of converting citric acid into acetyl-CoA, DLAT can control the citric acid cycle-oxidative phosphorylation pathway, thus affecting the energy supply of cancer cells (37). In gastric cancer cells, DLAT expression was significantly upregulated (38), making it a potential therapeutic target. DLAT promotes the growth of cancer cells by activating the pentose phosphate pathway (39). Alternol, a compound that binds to multiple Krebs cycle enzymes, inhibits

mitochondrial respiration and ATP production. This discovery offers a novel therapeutic strategy for treating prostate cancer (40).

PDHA1 AND PDHB

PDHA1 and PDHB encode subunits of the pyruvate dehydrogenase complex (PDC), an essential enzyme complex within the mitochondria responsible for catalyzingpyruvate oxidation to acetyl-CoA, connecting glycolysis and the tricarboxylic acid (TCA) cycle.

PDHA1 inhibition can increase proliferation, glycolysis, and Warburg effect in certain cancer cells. Gastric cancer has been shown to down regulate PDHA1, and elevated expression of PDHA1 correlates with poor prognosis (41). Down regulation of PDHA1 promotes the growth of gastric cancer. Exosomal miR-21-5p suppresses PDHA1 expression, thereby promoting glycolysis and cell proliferation in gastric cancer cells. PDHA1 expression in gastric cancer samples is negatively correlated with miR-21-5p levels(41). Additionally, miR-21-5p/PDHA may influence ovarian cancer drug resistance through exosomal miR-21-5p-mediated regulation of PDHA1 expression (42). The knockout strains had increased glycolysis, glucose intake, and glutamine consumption, while OXPHOS was inhibited, indicating enhanced Warburg effect and PDHA1. Furthermore, the proliferative capacity, angiogenic capacity, and drug resistance of the knockout esophageal cancer cells were significantly improved(43). PDHA1 is closely associated with prostate cancer growth, where it is involved in mitochondrial lipid synthesis. Therefore, PDHA1 may be useful as a therapeutic target for prostate cancer(44).

PDHB also acts as a cancer suppressor gene. PDHB over expression inhibits colon cancer cell proliferation, invasiveness, and glycolysis as it targets miR-146b-5p at the 3'-UTR end of the gene, promoting cancer cell growth (45). On the other hand, gastric cancer cells over expressing PDHB exhibit reduced proliferation and migration(46). PDHB inhibitors have also been shown to suppress cancer growth in various studies. For instance, reduced PDHB expression in non-small cell lung cancer indicates poor

prognosis for patients (47), while PDHB may serve as a biomarker for breast cancer(48). Thus, the progress made in the research on PDHA1 and PDHB in cancer highlights the broad potential applications of therapeutic drugs targeting these molecular targets.

MTF1

Metallothionein (MTF1) plays a crucial role in the treatment resistance of malignant cancers (49). Cells stimulated with heavy metals, such as copper, trigger the production of products encoded by MTF1,leading to the induction of metal sulfur production. During tumor biogenesis and progression, co-expression of proteins and other genes involved in metal homeostasis is implicated. Notably, MTF1 is highly expressed in ovarian cancer tissues, and its high expression is associated with poor patient survival and disease recurrence(50). MTF1 knockout can inhibit the EMT (epithelial-mesenchymal transition) process of ovarian cancer cells, thereby suppressing their proliferation, migration, and invasion, indicating that MTF1 may serve as a novel biomarker and therapeutic target for ovarian cancer (49). Given the multiple aspects of MTF-1 activities, monitoring changes in its expression and activity during cellular stress and cancer may prove valuable for cancer screening and prognosis studies.

GLS

GLS encodes Mitochondrial glutaminase K, which is dysregulated in many cancers. GLS can modulate promoter methylation modification and influence the clinical prognosis. In both *in vitro* and *in vivo* studies, GLS-targeted therapy has demonstrated its potential to inhibit cancer growth (51, 52). Similarly, GLS has been detected in clinical samples from breast cancer, esophageal cancer, head and neck cancer, and leukemia. The expression of GLS is associated with poor prognosis in statistical analysis.Therefore, GLS can be considered a prognostic biomarker for certain types of cancer(53). However, its use as a prognostic biomarker remains controversial and further research is necessary to clarify its role and potential clinical applications (54).

CDKN2A

During cancer development, aberrant gene silencing is highly associated with cell cycle regulation. Dysregulation of CDKN2A, which encodes the p16INK4a protein, has been causally linked to the pathogenesis of various cancer types, contributing to cancer recurrence, poor prognosis, cancer genesis, and metastasis(55). CDKN2A mutations are responsible for approximately 20-40% of familial cancers and 2-3% of sporadic melanomas(56). Moreover, non-synonymous mutations of CDKN2A were found in approximately 16% (9/56) of cutaneous melanoma metastases(57).Activation of CDKN2A has been reported in 95% of pancreatic adenocarcinoma cases due to promoter hypermethylation(58). In lung cancer, CDKN2A inactivation has been observed in 75% of cases (30/40), including 16 homozygous deletions, 10 methylations, and 4 mutations(59). CDKN2A gene mutations and abnormal methylation have also been reported in ovarian, gastric, and colorectal cancers, among others(55). Reactivating CDKN2A genetically and epigenetically could offer promising approaches for cancer prevention and treatment.

DISCUSSION

Copper ion concentration in the human body is tightly regulated by a homeostatic mechanism to maintain trace levels, as excess copper becomes toxic and leads to cell death. However, the mechanism underlying copper-induced cytotoxicity is still unclear (61, 62). Recently, a novel form of cell death,cuproptosis, was discovered, which operates independently of known cell death mechanisms(1). Cuproptosis-related genes were identified using CRISPR-Cas9 Loss-of-function screens, which revealed seven positively-regulated and three negatively-regulated genes.

So far, the identified copper ionophore-induced death genes include dihydrolipid amide dehydrogenase (DLD), fatty acylated protein targets pyruvate dehydrogenase (PDH) complex including DLAT, PDHA, and PDHB. While studies on these genes in cancer have been more extensive (3), other components of the lipoic acid pathway, such as fatty acyl synthase (LIAS) and FDX1, remain relatively understudied in cancer, and

further experiments are needed to verify their roles in different cancer types (1, 3). Notably, high cuproptosis activity status has been found to be a good prognostic indicator.

While some progress has been made in utilizing other types of programmed cell death for cancer treatment, there are still limitations in their application. Cuproptosis, being a novel form of programmed cell death, offers new perspectives on the correlation between its related genes and cancer prognosis. The combination of cuproptosis-targeted molecular drugs with existing therapies might open up new avenues for cancer treatment.

Currently, cuproptosisresearchis still in its infancy, and the existence of other signaling pathways for cell cuproptosis is not yet clear.Additionally, existing copper agents have poor targeting specificity and can cause serious side effects in patients undergoing treatment. These limitations and deficiencies impede the development and clinical implementation of cancer treatment strategies based on cuproptosis mechanisms.

In the future, researchers should focus on improving our understanding of the mechanism of cuproptosis in cancer cells and conducting thorough investigations into relevant mechanisms. Additionally, efforts should be directed towards developing copper-related formulations with high targeting and specificity (such as targeted nano-drug delivery systems) to maximize the targeting of cancer treatment while reducing toxic side effects. Lastly, it is necessary to develop and improve copper treatment plans in clinical practice in order to conduct relevant clinical trials and treatments for patients with cancer.

CONCLUSION

Cuproptosisis triggered by the direct interaction of copper ions with the fatty acylated components in the citric acid cycle of mitochondrial respiration. This interaction results in the aggregation of fatty acylated proteins and subsequent down regulation of iron-sulfur cluster proteins, leading to protein toxic stress and, ultimately

leading to cell death (Figure 3). The elucidation of this mechanism provides a clear understanding of howprevious copper ion drugs exert their anti-tumor effects. This provides potential possibilities for the clinical application of these drugs in anti-tumor therapy and also broadens the path for the development of new drugs targeting copper in the future.

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