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New Targets for Treating Cancer: Copper Death and Copper Accumulation in Cancer Cells

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Abstract: The accumulation of copper ions plays an important role in the apoptosis of cancer cells. Definition of copper death: Copper death (Cuproptosis) is a new form of programmed cell death caused by excessive copper. Copper ions can participate in multiple cellular signaling pathways and promote the growth and division of cancer cells. Copper ions are involved in angiogenesis, providing nutrients and oxygen for cancer cells and supporting the growth and spread of tumors. Together, they promote the progression of cellular inflammation. Copper ions are related to tumor metastasis and play a certain role in the migration and invasion of cancer cells.

Keywords: Copper Death; Tumor Cells; Mitochondrial Lipoylation.

1. Introduction

Mitochondria play a key role in normal cells, including generating energy (generating adenosine triphosphate, ATP through oxidative phosphorylation), regulating intracellular calcium homeostasis, participating in the regulation of apoptosis, and generating reactive oxygen species (ROS).

During tumorigenesis and development, the function and structure of mitochondria often change. On the one hand, for the energy requirements of their rapid proliferation, the energy metabolism of mitochondria in tumor cells undergoes reprogramming. For example, some tumor cells may prefer glycolysis to generate energy rather than relying on mitochondrial aerobic respiration, but mitochondria still play a role in providing some energy and metabolic intermediates. On the other hand, ROS produced by mitochondria have a dual role in tumorigenesis. Appropriate amounts of ROS can act as intracellular signaling molecules to promote the activation of signaling pathways related to cell proliferation and survival; however, excessive ROS can lead to cell damage and apoptosis. Tumor cells regulate the function of mitochondria to maintain a moderate level of ROS for their own survival and proliferation [1].

Furthermore, the apoptotic pathways involving mitochondria are often affected in tumors. Tumor cells can suppress mitochondria-mediated apoptosis through various mechanisms, such as altering mitochondrial membrane permeability and regulating the expression of apoptosis-related proteins, thereby evading the normal apoptotic program of cells and achieving unlimited proliferation.

2. The Relationship between Tumor Cells and the Energy Produced by Intracellular Mitochondria

The growth proliferation and survival of tumor cells are closely related to the acquisition and utilization of energy. Normal cells usually produce energy efficiently through aerobic respiration to meet their physiological functions and metabolic needs. However, tumor cells often show different characteristics in energy metabolism compared to normal cells [2].

Under aerobic conditions, tumor cells often tend to enhance the glycolysis pathway to obtain energy. Even with sufficient oxygen supply, they do not completely rely on aerobic respiration. This phenomenon is called the "Warburg effect". Although glycolysis is relatively inefficient in generating energy, it is fast and can quickly provide the energy and metabolic intermediates required for the rapid proliferation of tumor cells. In addition, tumor cells also increase the uptake and metabolism of amino acids such as glutamine to supplement the substances needed for energy and the synthesis of biological macromolecules[3].

To meet the ever-increasing energy demands, tumor cells also change the function of mitochondria. The energy production of mitochondria in tumor cells may be inhibited to a certain extent, but it still plays a role in providing a certain amount of ATP and participating in metabolic regulation[4]. The alterations in the energy metabolism of tumor cells not only support their rapid proliferation but also affect their response to treatment. Some therapeutic strategies targeting tumor energy metabolism are under investigation, aiming to inhibit tumor growth by interfering with the energy acquisition and utilization of tumor cells [5] .In conclusion, the unique energy metabolism mode of tumor cells is an important basis for their malignant biological behaviors. In-depth research on it is helpful for the development of new tumor treatment methods[6].

3. Cuproptosis Leads to the Death of Tumor Cells

Cuproptosis is a novel copper-dependent form of cell death, and the cuproptosis of tumor cells mainly involves the following processes: The homeostatic regulation of intracellular copper ions: Copper is a cofactor of essential enzymes in the human body. In tumor cells, copper ions are usually maintained at extremely low levels. After copper is transported to the cell surface via the blood system, it is catalyzed and reduced by the membrane surface STEAP protein to Cu+, which is more cytotoxic[7] . The copper ions that enter the cell pass through the outer and inner

mitochondrial membranes via COX17 and SLC25A3 in sequence and enter the mitochondrial matrix. At the same time, the copper ions in the cytoplasm can also bind to copper ion chelators (such as GSH and MT) to neutralize the cytotoxicity of copper [8],or be carried by the copper ion chaperone CCS to SOD1 to regulate the intracellular reactive oxygen species balance. The dysregulation of copper homeostasis leads to cell death: When copper ions accumulate excessively due to ionophores or transporters, on the one hand, FDX1 reduces Cu^{2+} to the more toxic Cu^{+}, inhibits the synthesis of mitochondrial respiratory-related iron-sulfur cluster proteins (Fe-S Cluster), causing protein toxic stress responses, and ultimately leading to cell death [9-11],On the other hand, as an upstream regulator of protein lipoic acidylation modification, FDX1 participates in regulating the lipoic acidylation of DLAT. Cu^{2+} can directly bind and induce the heteropolymerization of DLAT. The increase of insoluble DLAT will cause cellular protein toxic stress and induce cell death.

The main methods to trigger cuproptosis in tumor cells are to increase the intracellular free copper ion concentration. Specific ways include: overexpressing the copper ion transporter SLC31A1 to uptake more copper ions; knocking down ATP7B to reduce copper efflux, thereby regulating the intracellular copper ion concentration; using copper ion carriers (such as Elesclomol and Disulfiram) to directly transport extracellular Cu^{2+} into the cells; using buthionine sulfoximine (BSO) to deplete the endogenous intracellular copper chelator glutathione (GSH) to prevent it from chelating free copper ions[12-14]. The detection indicators of cuproptosis mainly include morphological observations (such as plasma membrane rupture, mitochondrial rupture, endoplasmic reticulum damage, and chromatin rupture), detection of related markers (such as Fe-S cluster proteins FDX1 and LIAS are markers of cuproptosis, the reduction of lipoylation of DLAT and DLST, and the increase in HSP70 levels), and detection of metabolic indicators (such as copper ion accumulation, accumulation of alpha-ketoglutarate, and reduction of succinate) [15].

Studies have shown that the proliferation and metastasis of tumor cells are more dependent on copper than normal tissues. The discovery of cuproptosis provides a new possible direction for tumor treatment, but related research is still ongoing and in-depth exploration. In practical applications, further research is needed on how to more effectively induce cuproptosis in tumor cells while reducing the impact on normal cells to achieve more precise and effective tumor treatment [16-19].

4. Cuproptosis and Copper Ions

Cuproptosis is a newly discovered form of cell death that is closely related to copper ions. The imbalance of intracellular copper ion homeostasis can lead to cuproptosis. Usually, the intracellular copper concentration is maintained at an extremely low level to prevent toxicity to the cells. Copper ions are cofactors of essential enzymes in the human body, and the same is true in cancer cells. When copper ions accumulate excessively due to ionophores or transporters, the following processes may be triggered to cause cuproptosis [20]. Inhibition of iron-sulfur cluster protein synthesis: fdx1 (a reductase) reduces Cu^{2+} to the more toxic Cu^{+}, inhibiting the synthesis of iron-sulfur cluster proteins related to mitochondrial respiration, causing a protein toxic stress response [21]. Induction of abnormal aggregation of related proteins: fdx1, as an upstream regulator of protein lipoic acidylation modification, participates in regulating the lipoic acidylation of dlat (one of the components of the pyruvate dehydrogenase complex) [22]. Cu^{2+} can directly bind and induce the heteropolymerization of dlat. The increase in this insoluble dlat leads to cellular protein toxic stress and induces cell death. In tumor treatment research, cuproptosis can be triggered by increasing the intracellular free copper ion concentration, such as overexpressing the transporter slc31a1 that uptakes copper ions, knocking down atp7b that exports copper ions, using copper ion carriers to transport extracellular Cu^{2+} into the cells, and using buthionine sulfoximine to deplete the endogenous intracellular copper chelator glutathione[23].The excessive accumulation and abnormal function of copper ions are the key factors leading to cuproptosis. In-depth research on the mechanism of cuproptosis is helpful for further understanding the process of cell death and providing new ideas and targets for the treatment of related diseases [24].

Changes in Mitochondria and Cuproptosis of Tumor Cells,The homeostatic regulation of intracellular copper ions plays an important role in the cuproptosis of tumor cells. Usually, copper ions are transported via the blood system and, after being delivered to the cell surface, are catalyzed and reduced by the membrane surface STEAP protein to the more toxic Cu^{+}. The copper ions that enter the cell pass through the outer and inner mitochondrial membranes via COX17 and SLC25A3 in sequence and enter the mitochondrial matrix. At the same time, the copper ions in the cytoplasm can also bind to copper ion chelators such as GSH (glutathione) and MT to neutralize the cytotoxicity of copper, or be carried by the copper ion chaperone CCS to SOD1 to regulate the intracellular reactive oxygen species balance [25-28].

When copper ions accumulate excessively due to ionophores or transporters, the following changes occur, leading to cuproptosis: On the one hand, FDX1 reduces Cu^{2+} to the more toxic Cu^{+}, inhibits the synthesis of iron-sulfur cluster proteins (Fe-S Cluster) related to mitochondrial respiration, causes a protein toxic stress response, and ultimately leads to cell death [29] On the other hand, FDX1, as an upstream regulator of protein lipoic acidylation modification, participates in regulating the lipoic acidylation of DLAT. Cu^{2+} can directly bind and induce the heteropolymerization of DLAT. The increase in this insoluble DLAT leads to cellular protein toxic stress, thereby inducing cell death. [30-31]

The detection indicators related to cuproptosis mainly include morphological observations (such as plasma membrane rupture, mitochondrial rupture), detection of related markers (such as Fe-S cluster proteins FDX1 and LIAS are markers of cuproptosis, the reduction of lipoylation of DLAT and DLST, and the increase in HSP70 levels) and detection of metabolic indicators (such as copper ion accumulation, accumulation of alpha-ketoglutarate, and reduction of succinate). [32] In the research of cuproptosis in tumor cells, some cuproptosis-related genes have been identified. For example, PDHA1 (a subunit of the pyruvate dehydrogenase complex) is a prognostic risk factor, and its expression is related to the progression and treatment sensitivity of tumors. PDHA1 is aberrantly expressed in many cancers, is closely related to prognosis, and is related to chemotherapy and immunotherapy. Knocking down PDHA1 can inhibit the proliferation and invasion of tumor cells [33, 34].

Studying cuproptosis provides a new direction for tumor treatment. Cuproptosis can be triggered by increasing the intracellular free copper ion concentration, such as overexpressing SLC31A1 that uptakes copper ions, knocking down ATP7B that reduces copper efflux, using copper ion carriers to transport extracellular Cu^{2+} into the cells, and using buthionine sulfoximine to deplete the endogenous intracellular copper chelator glutathione [35]. In conclusion, in-depth research on the changes in mitochondria and cuproptosis is helpful for further understanding the death mechanism of tumor cells and provides a basis for the development of new tumor treatment methods. However, related research is still ongoing, and specific therapeutic applications still require more experiments and clinical validations. [36] Promoting Copper Accumulation in Tumor Cells to Accelerate Their DeathPromoting copper accumulation in tumor cells to accelerate their death is a new direction in tumor treatment research. The following are some possible strategies. [37]

5. Cuproptosis and Copper Ion Carriers

Copper ion carriers can transport extracellular copper ions into cells, increasing the intracellular copper ion concentration. For example, elesclomol is an effective copper ion carrier and has shown potential in some experimental studies to promote copper accumulation in tumor cells and induce tumor cell death [38]. Inhibiting copper ion efflux-related proteins - ATP7A and ATP7B are proteins responsible for excreting excess copper ions from cells. Inhibiting their functions through drugs or gene editing techniques can prevent tumor cells from excreting copper ions, leading to intracellular copper accumulation [39]. Enhancing the expression of copper ion uptake-related proteins for example, overexpressing proteins responsible for copper ion uptake, such as SLC31A1, can increase the uptake of copper ions by tumor cells and thereby promote copper accumulation [40]. Disrupting the intracellular copper ion balance mechanism There are some molecules regulating copper ion balance in cells, such as glutathione (GSH). Using drugs or other means to deplete GSH in cells will interfere with the chelation and storage of copper ions, resulting in the accumulation of more free copper ions. Combined therapy Combining strategies that promote copper accumulation with traditional methods such as chemotherapy, radiotherapy, or immunotherapy may produce a synergistic effect and enhance the killing effect on tumor cells [41]. However, in practical applications, the specificity, safety, and possible side effects of these methods need to be fully considered. At the same time, due to the heterogeneity and complexity of tumor cells, the sensitivity of different types of tumors to copper accumulation may vary, requiring further in-depth research and individualized treatment plan design [42-44].

Apoptosis and Copper Metabolism, Apoptosis is a programmed form of cell death and plays an important role in both physiological and pathological processes. There is a certain association between copper metabolism and apoptosis [45]. Excessive copper ions can induce apoptosis through various mechanisms: Generating oxidative stress: As a redox-active metal, copper ions can participate in the Fenton reaction to generate reactive oxygen species (ROS). Copper-induced ROS can cause cytochrome C and mitochondrial apoptosis-inducing factor 1 to be released from mitochondria into the cytoplasm, causing caspase activation and DNA fragmentation. ROS also increases lipid peroxidation and depletes glutathione, making cells more susceptible to oxidative damage [46-48]. Causing DNA damage: Copper accumulation in the nucleus can bind to DNA, leading to DNA damage in multiple biological systems. Its potential binding sites involve guanine and cytosine on opposite strands. It can also induce base substitution mutations by generating oxidative damage through interaction with DNA. Affecting proteasome function: Copper binding to sulfhydryl groups can inactivate sulfhydryl-containing enzymes such as proteasome subunits and inhibit the chymotrypsin-like activity of purified 20S proteasomes or 19S proteasome deubiquitinases. This provides a copper-based idea for targeting the ubiquitin-proteasome system in the treatment of human cancers [49].

In addition, copper ions are also related to other types of cell death, such as necroptosis, paraptosis, pyroptosis, ferroptosis, and cuproptosis, etc. Cuproptosis is a newly reported novel form of death caused by excessive copper, and its regulatory process is closely related to mitochondrial metabolism. Excessive copper directly binds to lipoylated proteins in the mitochondrial tricarboxylic acid cycle, leading to abnormal aggregation of lipoylated proteins and loss of iron-sulfur cluster proteins in respiratory chain complexes, causing protein toxic stress responses and ultimately leading to cell death. In conclusion, abnormalities in copper metabolism may affect the survival and death processes of cells [50].

6. Phospholipid Peroxidation in Partial Cellular Cuproptosis

During the process of cellular cuproptosis, phospholipid peroxidation plays an important role. After the accumulation of copper ions in cells, it will trigger a series of reactions leading to phospholipid peroxidation. Copper ions can interact with lipid molecules such as fatty acids to promote the occurrence of lipid peroxidation. Phospholipid peroxidation will destroy the integrity and stability of the cell membrane, resulting in increased permeability of the cell membrane, leakage of intracellular substances, and affecting the normal function of the cells [51]. At the same time, the products of phospholipid peroxidation may further trigger intracellular oxidative stress responses, causing damage to intracellular biological macromolecules such as proteins and nucleic acids. This copper ion-induced phospholipid peroxidation is a key link in the process of cellular cuproptosis, accelerating the process of cell death. In addition, phospholipid peroxidation may also affect intracellular signal transduction pathways, interfering with the survival and proliferation signals of cells, thereby promoting tumor cells and others to die. However, the specific mechanism and regulatory pathway of phospholipid peroxidation in cellular cuproptosis still need further in-depth study [52].

7. Cellular Cuproptosis May be a New Target for the Prevention and Treatment of Cancer

First of all, many cancer cells exhibit abnormal metabolism and signaling pathways, making them resistant to traditional treatment methods. Cuproptosis, as a newly discovered form of cell death, provides a completely new perspective for

cancer treatment. By inducing cuproptosis in tumor cells, the problem of drug resistance faced by traditional treatment methods can be avoided [53]. Secondly, studying the related mechanisms of cellular cuproptosis can reveal specific molecular targets. For example, proteins related to copper ion transport, metabolism, and regulation, or enzymes related to the phospholipid peroxidation process. Developing specific drugs or treatment strategies targeting these targets is expected to achieve precise strikes on cancer cells while reducing damage to normal cells [54]. Furthermore, since different types of cancer cells may differ in copper metabolism and sensitivity to cuproptosis, in-depth research can provide a basis for personalized cancer treatment. Tailored treatment plans based on the specific type of tumor and the genetic characteristics of individual patients can improve the treatment effect [55]. However, using cellular cuproptosis as a target for cancer treatment also faces some challenges. For example, the specific molecular mechanism of cuproptosis still requires more in-depth research and clarification to ensure the safety and effectiveness of treatment strategies. At the same time, how to precisely regulate the distribution and concentration of copper ions in the body and how to avoid adverse effects on normal tissues are also key issues that need to be addressed [56-57]. In conclusion, although cellular cuproptosis has great potential as a new target for cancer treatment, more research and exploration are still needed to transform it into a practical clinical treatment method.

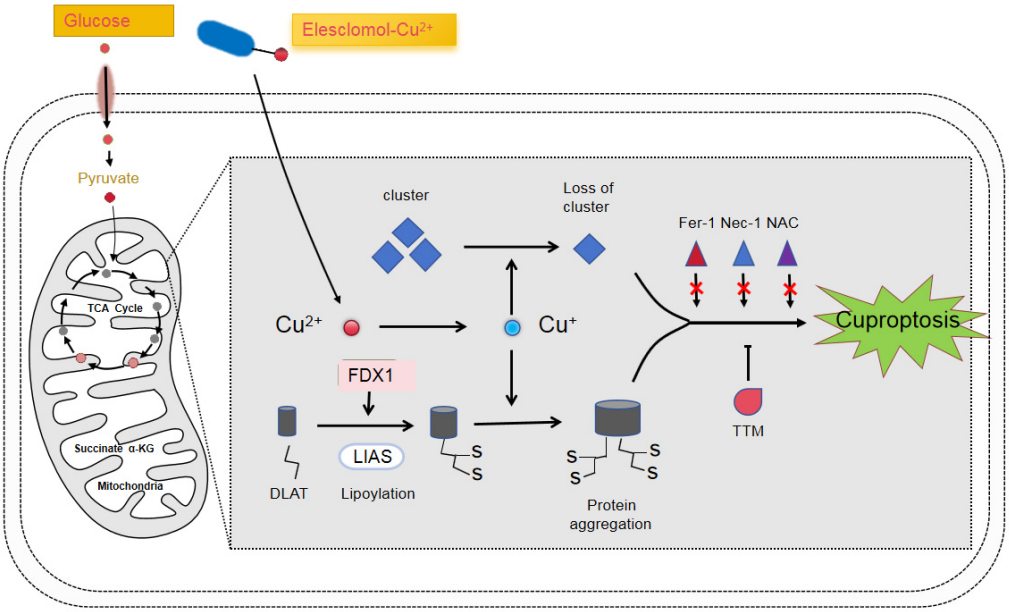

Figure 1. The Diagram of the Relationship between Cuproptosis Mitochondria and Copper Ions within Cells

Inhibiting Cell Phospholipid Peroxidation to Prevent the Generation of Cell Inflammation, Phospholipids are an important component of cell membranes, and their peroxidation can cause damage to the structure and function of the cell membrane. This can disrupt the barrier function of the cells, causing the leakage of intracellular substances and promoting the release of inflammatory factors [58]. When phospholipid peroxidation occurs, a series of reactive oxygen species and lipid peroxides are produced. These substances can activate intracellular inflammatory signaling pathways, such as the nuclear factor-κB (NF-κB) pathway. The activation of NF-κB can lead to an increase in the expression of multiple inflammation-related genes, further intensifying the inflammatory response [59]. By inhibiting phospholipid peroxidation, the generation of these harmful products can be reduced, thereby reducing the activation of inflammatory signaling pathways and inhibiting the release of inflammatory factors. This helps maintain the stability of the intracellular environment and prevent the occurrence and development of inflammation. Currently, there are multiple ways to inhibit phospholipid peroxidation. For example, using antioxidants such as vitamin E and vitamin C, which can directly scavenge free radicals and reduce the occurrence of phospholipid peroxidation. In addition, some drugs and natural compounds have also been found to have the effect of inhibiting phospholipid peroxidation, but their specific mechanisms of action and clinical applications are still under further study.

8. Summary and Outlook

Nanomaterial-Induced Cuproptosis in Tumor Cells: A variety of nanomaterials have been developed and used to induce cuproptosis in tumor cells for cancer treatment. A large number of studies have shown that the combined use of cuproptosis and other tumor treatment methods has achieved better therapeutic effects, showing great potential. The combined use of cuproptosis and other tumor treatment methods may be a research direction in the future, such as combined use with immunotherapy, targeted therapy, to improve the therapeutic effect; Personalized medicine: Through genetic testing and analysis of patients, determine their sensitivity to cuproptosis to achieve personalized cancer treatment. New targets and drugs: Further research on the mechanism of cuproptosis may discover new targets and drugs, providing more options for cancer treatment. It should be noted that the current research on cuproptosis and cancer treatment is still in the early stage, and further research and clinical trials are needed to verify its effectiveness and safety.

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