

CLINICAL PERSPECTIVES ON CUPROPTOSIS: CURRENT UNDERSTANDING AND FUTURE DIRECTIONS

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Abstract:

Copper ions, vital micronutrients in nature, play a crucial role in maintaining organismal homeostasis, from microorganisms to humans. In the human body, copper serves as an enzyme cofactor, influencing essential processes such as blood coagulation, hormone maturation, and energy metabolism. Additionally, copper actively engages in various biological pathways. However, the delicate balance of copper homeostasis can be disrupted, causing detrimental effects. Copper overload, for instance, leads to the aggregation of fatty acylated proteins and destabilizes Fe-S cluster proteins, resulting in protein-related stress and, ultimately, cellular demise. This cascade of events adversely impacts the proper functioning of multiple organs. Understanding the intricate role of copper in both maintaining health and causing harm is imperative. This study delves into the multifaceted aspects of copper ions in biological systems, shedding light on the mechanisms underlying their beneficial effects as micronutrients and the harmful consequences of copper overload.

Keywords: Copper ions, Homeostasis, Enzyme cofactor, Protein aggregation, Cellular toxicity.

Introduction

In nature, from small microorganisms to large animals, plants and humans, copper ions are beneficial and necessary for the homeostasis of organisms as an essential micronutrient. In the human body, as a cofactor of enzymes, copper homeostasis contributes to blood coagulation, hormone maturation and energy processing, and also participates in some biological processes. Conversely, copper overload leads to the aggregation of fatty acylated proteins and the instability of Fe-S cluster proteins, which leads to toxic stress of proteins and eventually cell death, resulting in abnormal work of multiple organs.

1. The basic concept and brief history of copper death

Cuproptosis, also known as copper ptosis, is a new type of cell death caused by cytotoxicity or even cell death caused by intracellular copper overload. There are four evolutionarily conserved lipidylated proteins in mammals, alpha-ketoglutarate dehydrogenase (KDH) and pyruvate dehydrogenase (PDH) directly involved in mitochondrial TCA cycle; branched-chain alpha-ketoacid dehydrogenase (BCKDH), which is involved in the decarboxylation process of branched-chain amino acid catabolism; glycine cleavage system (GCV) for Glycine and Serine catabolism^[1]. The copper death process occurs through the direct binding of copper to the above-mentioned fatty acylated proteins. Fdx1 and Fdx2 are known to be two ferredoxin proteins in human cells that are sequence orthologs to yeast Yah1, but the latter is functionally complementary to Yah1. Yah1 is associated with human mitochondrial myopathy^[2]. Vallières C, Holland SL, Avery SV^[3] found that the relatively conserved evolutionary mitochondrial ferredoxin is an important target of copper toxicity in cells, and it has been verified in bacterial and eukaryotic mitochondria.

Peter Tsvetkov, as the first author, has published the advantages of copper carriers (ilolimus and disulfiram) carrying copper ions into cells in the treatment of drug-resistant cancer cells in 2019; On the basis of the above, Tsvetkov P, Coy S, Petrova B, et al.^[4] In 2022 further revealed that copper death is mediated by mitochondrial regulation of

copper ionophore induced cell death, and FDX1 (ferredoxin 1, Direct site of action of copper ionophore), as upstream regulators of protein lipid acylation, play a key role in Cu ionophore induced cell death. Figure 1 briefly summarizes the important events related to copper-induced cell death. This concept is significantly different from the programmed cell death pathways such as apoptosis, non-apoptotic (autophagy, pyroptosis, ferroptosis, endogenous death) that have been proposed, and copper death cannot be blocked by inhibitors of the above pathways.

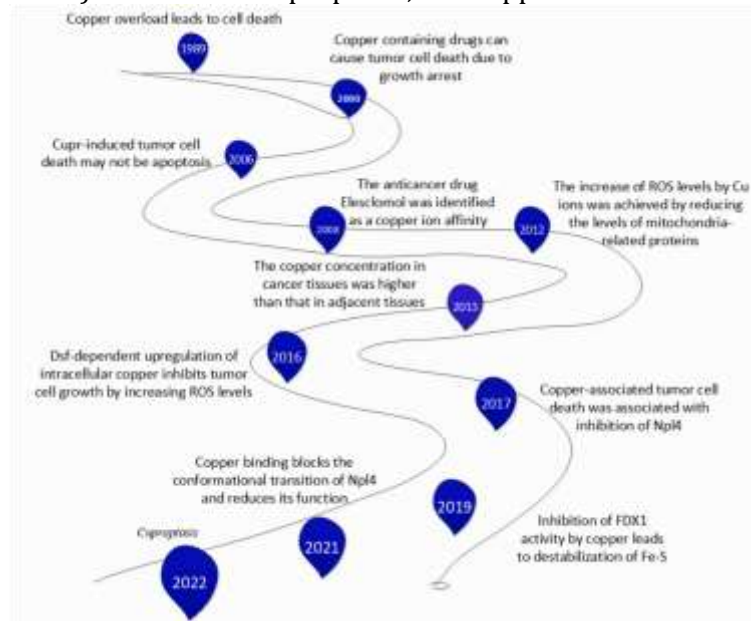


Figure 1: Timeline of copper death discovery

3. Common copper homeostasis related diseases in clinic

3.1 Copper stable hereditary diseases: Wilson disease, Menkes disease

Under normal circumstances, the human body obtains divalent copper ions required by the ingestion of high-copper foods such as shellfish, various animal viscera, blood, and bean foods. Cu^{2+} binds to the divalent metal transporter DMT1 on the membrane and is converted to Cu^{+} under the action of reductase. The intracellular copper content is regulated by active copper input SLC31A1 (CTR1)^[5] and copper transport enzymes ATP7A and ATP7B, so as to maintain the copper content at a relatively low level, which can not only play a normal biological role, but also prevent cell damage caused by copper overload. However, once genetic variation occurs in copper homeostasis, it will endanger human life in severe cases ^[6].

3.1.1 Copper accumulation liver disease

Mutations in the copper export ATP7B gene cause copper accumulation in the liver and neurons, leading to a variety of diseases, such as hepatolenticular degeneration (HLD), also known as Wilson's disease (WD)^[7]. It is a rare autosomal recessive genetic disorder of copper metabolism with multiple families in China. The pathogenic gene is 13q14.3. The ATP7B enzyme encoded by the mutant gene cannot transport intracellular copper to the Golgi apparatus and vesicles, and the unbound ceruloplasmin (CP) released into the serum increases and is decomposed. In addition, the secretion of bile is reduced, and intracellular copper overload is deposited in the liver, nervous system and other organs, causing irreversible copper cell death.

In patients with Primary biliary cholangitis (PBC), viral hepatitis and primary liver cancer, copper particles are deposited in the body, but it is rare ^[8]. Timm silver staining with high specificity and easy to distinguish can greatly improve the diagnosis rate of copper accumulation diseases, which is convenient for clinical application ^[9].

3.1.2 Copper deficiency diseases, such as Menkes disease (MD) and CTR1 (also known as SLC31A1) deficiency

Studies have shown that Menkes disease can cause loss of copper absorption and utilization function after mutation of ATP7A gene encoded by the pathogenic gene Xq21.1. It is a hereditary copper deficiency that occurs in infancy with fatal risk, and is characterized by death caused by progressive nerve damage. Similar to the intestinal epithelial cells, CTR1 and ATP7A at other absorption sites cooperate to regulate Cu content and transport direction. When CTR1 is dysfunctional, clinical symptoms associated with Cu ions will be observed.

3.2 Copper accumulation is associated with neurodegenerative diseases

The role of copper ions in normal brain development.

Copper participates in the myelination of central cerebral cortex and peripheral spinal cord, affects the excitability of neuronal synaptic activity and signal transduction, and regulates neuronal death. The brain is the second largest organ containing copper after the liver. CTR1 is responsible for the entry of copper into the cell, and ATP7A is responsible for the transport of copper out of the cell.

Studies have shown that high-copper diet can cause abnormalities in a variety of neurobehavioral function indexes in rats, and the decreased expression of MAP2 and GAP43 at the synaptic interface of neurons in the hippocampus may be involved in the occurrence of learning and memory impairment in neurobehavioral function^[10].

Copper accumulation and Alzheimer's disease, Parkinson's disease.

It is worth noting that copper accumulation in different parenchymal parts of the brain can affect normal brain function, such as dorsal striatum ^[11] and hypothalamus ^[12]. In addition, as the structural medium of blood-brain barrier (BBB), the increase of copper content in brain microvessels will further aggravate brain parenchymal damage. Studies by Tiflany-Castiglioni et al ^[13] and Rivera-Mancia et al ^[14] found that copper overload induced the occurrence of several neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Amyloid plaque deposition in Alzheimer's disease and protein aggregation of α -synuclein in Parkinson's disease are both associated with altered copper balance^[15].

3.3 Regulate your sleep

The results of studies on sleep duration and serum copper are inconsistent. Researchers have found that regulating the status of copper by gene knockout can affect sleep quality ^[16], and it is speculated that it is related to the impaired activity of dopamine β -hydroxylase required for norepinephrine secretion in the locus coeruleus ^[17-18] because copper ions are required for the normal physiological function of this enzyme. In addition, the researchers confirmed the distribution of copper ions in the site with the binding site.

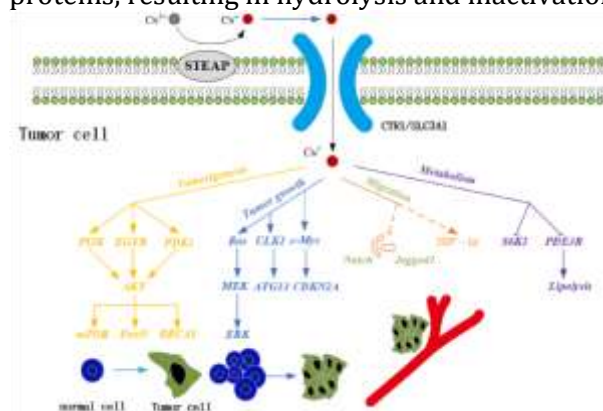
3.4 Tumor proliferation

Studies have confirmed that compared with normal subjects and non-dividing cells, the copper ion content in serum and tumor pathological tissues of tumor patients is higher ^[19-20]. Local increase of radioactive Cu²⁺ has also been detected in a variety of animal cancer models, which has auxiliary diagnostic significance ^[21].

In addition, purple potato is rich in anthocyanins with strong antioxidant activity, which is used as a source of anthocyanins because of its wide source and high economic value. Elesclomol is a small molecule anticancer drug that targets FDX1 to induce copper death. As a key regulator of copper death, FDX1 can affect the biosynthesis of Fe-S cluster proteins on the one hand, and oxidize Cu²⁺ to reduce Cu on the other hand. Guo Ling et al.^[22] purified and extracted anthocyanin components mainly composed of cyanidin and its derivatives from purple potato, and used molecular docking and other targeted techniques to find that the extract was very compatible with Fe-S cluster protein and FDX1 protein molecular docking, and negatively regulated their expression to exert copper death, and the action site was very similar to that of anticancer drugs. This finding undoubtedly provides a good news for the clinical treatment of leukemia patients.

In the process of tumor evolution, researchers also found that the increase of copper content promoted the angiogenesis of tumor cells and accelerated their proliferation and metastasis ^[23], and found that copper was involved in multiple signal transduction pathways in tumor cells, as shown below. After inducing the generation of

oxygen free radicals in the body and destroying the phosphodiester bond, copper complexes interact with DNA and proteins, resulting in hydrolysis and inactivation of DNA [24].



Tumorigenesis: Copper directly binds or activates PI3K, EGFR, or PDK1.

Tumor growth: Copper affects mitogen-activated protein kinase MAPK (such as binding to MEK and promoting downstream ERK phosphorylation) and autophagy pathways (UNC-51-like kinase (ULK1)) or indirectly alters c-Myc stability.

Promote vascular tumor migration: Copper ions indirectly inhibit Notch pathway ligand Jagged1 or indirectly promote HIF- α .

Regulation of tumor metabolism: Copper can also regulate PDE3B (phosphodiesterase 3B) or S6K1 (ribosomal protein S6 kinase β 1).

Figure 2: Copper and cancer signaling pathways

4. Conclusions

Copper ionophore and copper chelator have high copper ion binding rate, which can hinder the high demand for copper of abnormal dividing cells. Both of them can provide ideas for the development of adjuvant therapeutic drugs for tumors.

Given that the targets of copan-induced toxicity in human cancer cells, including lipoacylation and Fe-S cluster proteins, are conserved evolutionarily from bacteria to humans, this suggests the possibility of copdiolysis being exploited by microorganisms that naturally synthesize copper ionophores to exhibit antimicrobial activity?

A number of studies have pointed out that trace elements of copper have been detected in whole blood samples of COVID-19 patients during the global pandemic in 2020, suggesting that the level of copper may have certain guiding significance for the prognosis of patients. However, there is no consensus on whether patients with low copper can be treated with copper supplementation.

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