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Research Progress on Mechanism of Chinese Medicine Intervention on Cuprotosis Induced Apoptosis of Tumor Cells

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Abstract: In the current field of cancer treatment, the search for new therapeutic targets and methods remains a focal area of research. Traditional Chinese medicine contains a variety of pharmacologically active components that interact with multiple targets and signaling pathways in the human body, demonstrating potential anti-tumor effects. Copper plays a crucial role in various physiological processes; its homeostasis is tightly regulated to limit the production of oxidative stress and toxicity, and it intervenes in the processes of tumor development, metastasis, and recurrence. Cuproptosis represents a novel form of copper-dependent cell death that differs from previously known regulatory cell death mechanisms, offering a fresh perspective on the link between copper homeostasis and mitochondrial metabolism. Targeting copper has emerged as a new anti-cancer strategy. Current research on the intervention of cuproptosis by TCM is limited; leveraging the advantages of TCM's multi-target, multi-pathway approach could further elucidate the mechanisms of cuproptosis, bringing more possibilities to cancer treatment modalities.

Keywords: Cuprotosis; Chinese Medicine; Tumor Cells; Apoptosis; Action Mechanism.

1. Introduction

Over the past few decades, tumor treatment methods have remained largely unchanged, primarily involving surgical removal of the affected area and multi-drug combination chemotherapy. However, the survival rate for patients with persistent metastasis remains poor [1]. Therefore, the search for new treatment methods has become a hot topic in current research. Copper, an essential trace element within cells, plays a crucial role in maintaining vital activities. Copper ions can influence important biological processes such as cell cycle regulation, DNA damage repair, and oxidative stress responses. In the occurrence and development of cancer, copper has been shown to play a significant role. Existing studies have already revealed the important roles of ferroptosis, autophagy, apoptosis, and pyroptosis in tumors. However, research on the mechanism of Cuprotosis in tumors is relatively scarce. Thus, an in-depth exploration of the mechanisms related to Cuprotosis could not only enrich our understanding of tumor biology but also provide a theoretical foundation and practical guidance for future cancer treatment, prognosis evaluation, and the development of targeted drugs. Additionally, studies have indicated that traditional Chinese medicine shows potential in regulating copper homeostasis, participating in oxidative stress pathways, and modulating apoptotic pathways. Based on these effects, using traditional Chinese medicine to regulate Cuprotosis, thereby inducing cancer cell death, has been regarded as a novel and effective therapeutic strategy.

2. From Copper-Induced Cell Death to Cuprotosis

In recent years, the connection between copper and programmed cell death has long been a focal point of interest. The concept that copper could induce cell death was first proposed in the 1980s, though its exact mechanism was not clearly elucidated at that time. Copper ion carriers are lipid-soluble molecules capable of reversibly binding copper ions and transporting them through the cell membrane or mitochondrial membrane structures. Both disulfiram, a copper ion carrier, and elesclomol, another copper ion carrier believed to possess cell-killing capabilities, have been shown to induce cell death[2].Reactive oxygen species (ROS), which primarily originate from mitochondria and play a key role in intracellular redox reactions, are critical in cell death mechanisms. In studies on elesclomol-induced cell death, researchers discovered that the reason elesclomol causes cell death is that various mitochondria-related factors increase ROS, which in turn induces apoptosis in cancer cells. These factors include the reduction of mitochondrial proteins and the oxidation of ascorbate. This suggests that the cell death is triggered by copper itself rather than the copper ion carrier [3]. A study conducted in 2013 on human leukemia K562 cells demonstrated that, following transport into the cells via elesclomol, copper ions were able to oxidize ascorbic acid and react with decomposition of hydrogen peroxide(H2O2), generating more destructive ROS [4]. In 2015, it was further discovered that elesclomol-Cu complexes could arrest cells in the G1 phase, cause DNA damage, and affect mitochondrial membrane potential. Elesclomol-Cu was also found to act directly on the mitochondrial membrane, resulting in changes to the membrane potential: low concentrations of elesclomol led to hyperpolarization, while higher concentrations caused depolarization [5]. In prior studies concerning copper-induced cell death, most researchers attributed this process to the impact of copper on mitochondrial function. However, in the investigation of elesclomol's mechanism of action, the use of the ROS inhibitor N-acetylcysteine did not mitigate the cytotoxic effects of elesclomol-Cu, suggesting that ROS-

mediated cell death may not be the primary mechanism through which copper induces cell death.

It was not until March 2022 that Tsvetkov et al [6] discovered that, even when known cell death pathways were blocked, abnormally elevated intracellular copper ions could still induce cell death, which they termed "Cuprotosis". This mechanism involves the accumulation of intracellular copper ions, which bind directly to lipoylated components of the tricarboxylic acid TCA) cycle. This binding disrupts the regulation of protein lipoylation by ferredoxin 1 FDX1) and leads to the aggregation of lipoylated proteins and the destabilization of Fe-S cluster proteins. These molecular disruptions trigger toxic stress responses in proteins, ultimately resulting in cell death.

3. Copper Homeostasis in Tumor Cells

The association between copper and tumors was first identified through the discovery of elevated copper levels in many cancers. Studies on cancers such as lung cancer and prostate cancer have observed that serum copper ion levels in tumor patients are significantly higher compared to those in healthy individuals [7] [8]. The normal function of copper ions in cancer cells depends on the regulation of copper homeostasis and the interaction of various proteins. Copper ion transporter proteins CTR1 and ATP7B regulate the influx and efflux of copper ions in and out of the cell, while the transport of copper ions across the outer and inner mitochondrial membranes is controlled by cytochrome c oxidase-17 (COX-17) and the transmembrane transporter solute carrier family 25 member 3 (SLC25A3), respectively.

Copper ions that enter the cytosol and the intermembrane space of the mitochondria bind with glutathione (GSH) and metallothioneins to form copper-containing molecular chaperones. The transfer of copper to cytochrome c oxidase (COX) in the mitochondrial intermembrane space is mediated by cytochrome c oxidase assembly factor 6 (COA6), which is essential for maintaining normal cellular function [9].

(1) Copper Ion Transmembrane Transport

CTR1 (Copper Transporter 1) is the primary transporter responsible for cellular copper uptake, specifically for the transport of Cu+ ions. In tumor cells, copper ion uptake relies on the involvement of CTR1, which often exhibits elevated expression in tumors with increased intracellular copper levels. The upregulation or downregulation of CTR1 directly influences intracellular copper concentrations, and CTR1 also affects the rise in ROS and the decrease in adenosine triphosphate (ATP) levels caused by copper absorption [10]. High levels of CTR1 have been identified in prostate cancer, hepatocellular carcinoma, and pancreatic cancer [11][12]. Yu et al [13] discovered that inhibiting CTR1 and blocking copper uptake led to increased mitochondrial ROS levels and reduced ATP levels in pancreatic cancer cells, resulting in enhanced autophagy as a defense mechanism against cell death. In the bloodstream, Cu2+ is transported to the cell surface and reduced to monovalent copper under the catalytic action of STEAP proteins. Cu+ then binds to two His-Met-Asp clusters at the N-terminus of CTR1, where it is maintained in its reduced state for transport into the cell [14]. Once inside the cell, Cu+ is transported from the mitochondrial intermembrane space to the mitochondrial matrix via SLC25A3 [15]. ATPases, including ATP7A and ATP7B, are responsible for the efflux of copper ions. They regulate copper levels by transferring copper from the cytosol to the Golgi apparatus, and when copper levels are excessive, they relocate to the lysosome, where copper is released through vesicular excretion [16]. In some tumor cells, increased ATP7A levels facilitate copper efflux, protecting tumor cells from the damaging effects of copper-mediated oxidative stress [17].

(2) Interaction of Copper Ion Chaperones

Atox-1 is a copper chaperone that plays a critical role in maintaining copper homeostasis. It binds to copper ions via two cysteine residues and transfers them to ATP7A and ATP7B in the trans-Golgi network. This facilitates the synthesis of copper-dependent enzymes, such as glutathione oxidase, tyrosinase, and ceruloplasmin, thereby regulating intracellular copper concentration [18]. Studies have found that Atox-1 is upregulated in several types of cancer, including breast cancer, colorectal cancer, uterine cancer, and liver cancer [19]. Immunofluorescence studies show that Atox-1 expression at the leading edge of cells promotes tumor cell migration [20]. A recent study demonstrated that deletion of the Atox-1 gene significantly reduced single-cell migration speed and directionality, potentially due to the disruption of copper delivery to ATP7A[21] Copper chaperone for superoxide dismutase (CCS) delivers copper ions to superoxide dismutase 1 (SOD1), which detoxifies ROS and helps maintain copper homeostasis. CCS, in the presence of O_2, accelerates the formation of disulfide bonds in SOD1, which are essential for proper spatial conformation and enzymatic activity. SOD1 plays a crucial role in catalyzing the conversion of superoxide radicals into H2O2, maintaining intracellular ROS balance; its inactivation can lead to cell death [22].COX is a vital component of oxidative phosphorylation, and some mitochondrial copper chaperones are involved in the assembly and function of COX by storing or delivering copper ions [23]. COX is a mitochondrial respiratory chain enzyme that participates in ATP synthesis. COX17 is believed to play a role in the copper transport pathway to COX, transferring copper ions from the cytosol to the mitochondrial intermembrane space. Inhibition of COX17 may lead to reduced mitochondrial ATP levels and tumor cell death [24]. The absence of COA6 disrupts the functionality of respiratory complex IV. Under the influence of COA6, copper ions are transferred to COX, contributing to its proper assembly [25].

4. Molecular Mechanism of Cuprotosis

(1) Copper-Catalyzed Oxidative Stress

Oxidative stress is the result of an imbalance between oxidation and antioxidation within the body, often manifesting as elevated concentrations of ROS. This can lead to the death of normal cells or tumor cells. Studies have shown that copper can induce oxidative damage and apoptosis through two primary mechanisms:(1)Fenton Reaction: Copper ions can catalyze the Fenton reaction, producing highly reactive hydroxyl radicals. This reaction significantly increases ROS concentrations in tumor cells, leading to cell death. Copper ions also facilitate the H2O2 via the Fenton reaction, generating large amounts of ROS, which can directly oxidize and cleave DNA. This induces necrotic apoptosis and toxic damage in tumor cells. Simultaneously, copper ions deplete intracellular glutathione (GSH), converting it to glutathione disulfide (GSSG). This process reduces the cell's ability to eliminate ROS, further enhancing the Fenton effect and exacerbating oxidative stress, thereby increasing cytotoxicity. Li et al [26] demonstrated that copper-coordinated covalent organic frameworks (Cu-COF)

catalyzed ROS production from H2O2, causing significant DNA damage. In the Cu-COF treatment group, the DNA supercoiled form bands disappeared, and the number of nicked and linear DNA bands increased in a concentration-dependent manner. Additionally, copper ions can induce cancer cell death by promoting ROS production and regulating relevant signaling pathways. The copper ion carrier NSC319726 activates Cu2+, promoting ROS production, depleting deoxyribonucleotides, and causing DNA double-strand breaks and base oxidation. This triggers a DNA damage response, arrests cells in the G1 phase, and induces cell death [27]. Disulfiram, when combined with Cu2+, promotes ROS generation, activates the P38 signaling pathway, and inhibits the NF-kB signaling pathway, thereby inducing cancer cell death[28]. Copper ions also convert GSH to GSSG, reducing intracellular GSH levels, an essential antioxidant. This conversion disrupts the GSH antioxidant defense system, weakening its ability to scavenge highly reactive hydroxyl radicals, ultimately leading to tumor cell apoptosis.(2)Mitochondrial Apoptotic Pathway: After copper enters the cell, a portion is transported to the mitochondria, where it activates cytochrome c oxidase (CCO). However, excessive copper exposure inhibits the activity of antioxidant enzymes, leading to an overproduction of ROS. This triggers the activation of caspase, specifically cysteine aspartate proteases, which cleave deoxyribonuclease inhibitors, resulting in DNA fragmentation and disruption of the mitochondrial electron transport chain. Consequently, the mitochondrial apoptotic pathway is activated, compromising mitochondrial membrane integrity and increasing the expression of cytochrome c (CytC), apoptosis-inducing factors, endonuclease G, and apoptotic protease-activating factor 1, ultimately triggering cell apoptosis [29]. Furthermore, cells exposed to copper show upregulation of mitochondrial apoptosis-related factors. By regulating mitochondrial membrane potential and increasing membrane permeability, apoptosis factors are continuously released, accelerating the apoptosis process[30][31].

(2) Inhibition of Protease Activity in Cells

The ubiquitin-proteasome system (UPS) is a crucial protein degradation pathway in the human body, responsible for degrading approximately 80% of intracellular proteins. This system consists of two major processes: substrate protein ubiquitination and the subsequent degradation of ubiquitin-tagged proteins by the proteasome [32]. Ubiquitination plays an essential role in various cellular activities, such as apoptosis, signal transduction, and damage repair. The ubiquitination cascade requires the participation of three enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). The process begins with the formation of a thioester bond between the carboxyl terminus of ubiquitin and a cysteine residue on E1 (E1-Ub), thereby activating the ubiquitin molecule. The activated ubiquitin is then transferred to E2 ubiquitin-conjugating enzymes (E2-Ub). Finally, a substrate-specific E3 ligase transfers ubiquitin from E2 to specific substrate proteins [33] The proteasome is the core mechanism responsible for protein degradation in the UPS, predominantly located in the cytoplasm and nucleus of eukaryotic cells. It recognizes and degrades proteins that are no longer needed or are misfolded, breaking them down into short peptides or amino acids. These amino acids are then reused for new protein synthesis, helping to maintain intracellular protein homeostasis. Ubiquitin-labeled proteins can be recognized and degraded by the 26S proteasome or via the autophagy-lysosome pathway, or the ubiquitin tag can be removed by deubiquitinating enzymes (DUBs), thereby regulating downstream signaling pathways. Ubiquitination is central to the two major protein degradation pathways in cells: the 26S proteasome pathway and the autophagy-lysosome pathway. The 26S proteasome efficiently degrades short-lived, soluble, misfolded, or unfolded proteins and peptides, while the autophagy-lysosome pathway specializes in degrading long-lived proteins, insoluble protein aggregates, and malfunctioning organelles. Both pathways rely on ubiquitination to tag proteins for degradation, ensuring cellular homeostasis and proper protein function [34]. Copper complexes can mediate oxidative damage leading to cell death, and they also act as UPS inhibitors by reducing the stability of ubiquitin tags, inhibiting the activities of E1, E2, and E3 enzymes, and disrupting the linkage between ubiquitin molecules and target proteins, thus exhibiting antitumor effects [35]. Elevated intracellular Cu2+ levels can inhibit the peptidase activity of the isolated 20S proteasome, thereby impairing proteasome function. Studies have demonstrated that copper ions interfere with UPS functionality through various mechanisms. For example, the disulfiram-Cu2+ complex damages cancer cells by inhibiting proteasome activity and increasing ubiquitin-protein interactions. Additionally, the disulfiram-Cu2+ complex blocks upstream signaling of the proteasome system and inhibits ubiquitin-dependent ATPase, preventing the degradation of ubiquitinated proteins [36].

(3) Induction of Lipoylated Protein Aggregation and Inhibition of Mitochondrial Metabolic Function

A critical process necessary for copper-induced cell death is the lipoylation of proteins involved in ATP production [37]. In cuprotosis, protein lipoylation is a highly conserved post-translational modification in which lipoic acid is covalently attached to lysine residues via an amide bond. Lipoylated metabolic enzyme complexes are essential for maintaining normal mitochondrial function, as these enzymes regulate key metabolic complexes that control the entry of carbon into the TCA cycle. Lipoylation of these proteins is required for their enzymatic activity. Copper ions disrupt this system by binding to the lipoylated components, causing the aggregation of lipoylated proteins. This leads to mitochondrial metabolic dysfunction, triggering toxic protein stress and ultimately inducing cell death [38]. FDX1, an upstream regulator of protein lipoylation, has been identified as a key modulator of copper-induced cell death. FDX1 is involved in electron transfer, reducing Cu2+ to Cu+ within cells, which then binds to lipoylated proteins. This interaction promotes the aggregation of these proteins, disrupting their structure, function, and enzymatic activity, thereby impeding the normal progression of the TCA cycle. Studies have shown that the loss of lipoic acid synthase can significantly reduce copper's toxic effects [39]. Furthermore, knocking out FDX1 or enzymes related to lipoylation effectively eliminates protein lipoylation, rendering cells resistant to copper-induced death [40]. These strategies highlight the potential importance of regulating lipid metabolism pathways and protein modification in reducing susceptibility to cuprotosis. By interfering with the lipoylation process, copper induces mitochondrial stress, disrupts cellular energy production, and leads to the toxic accumulation of dysfunctional proteins, which together contribute to the cell death pathway unique to cuprotosis.

(4) Copper Ion Carriers Induce Cell Death

The accumulation of copper ions within cells disrupts the TCA cycle, causing aberrant aggregation of lipoylated proteins and reducing the levels of iron-sulfur cluster proteins. Copper ion carriers facilitate the transmembrane transport of copper ions, effectively delivering them to specific locations within tumor cells, significantly increasing intracellular Cu2+ levels and effectively inducing tumor cell death. In addition, the Cu-disulfiram complex has demonstrated potential as an adjuvant therapy when combined with established chemotherapeutic agents, targeting multiple pathways, including regulating ROS levels, affecting the UPS, and inhibiting the NF-κB pathway[41-43]. The anticancer mechanism of disulfiram appears to be copper-dependent, as it forms a complex with copper that allows it to specifically target cancer cells. The reaction between disulfiram and Cu results in the generation of extracellular ROS, which subsequently induces apoptosis in cancer cells [44]. Yoshino et al. found that disulfiram's metabolites accumulate in cancer cells, inducing ROS production and leading to apoptosis [45]. Elesclomol, an anticancer drug that targets mitochondrial metabolism, has long been understood to exert its anticancer effects by promoting the accumulation of intracellular ROS, ultimately inducing oxidative stress. This process involves multiple molecular targets, from mitochondrial metabolism to nuclear DNA [46]. The prevailing view is that elesclomol acts as an oxidative stress inducer, with excess ROS in cells potentially triggering apoptosis. Additionally, some studies have found that elesclomol-induced cell damage also involves DNA damage and cell cycle arrest [47]. Recent research has discovered that after treatment with elesclomol, copper levels selectively accumulate in mitochondria. It has been reported that elesclomol degrades ATP7A in colon cancer cells, leading to further copper ion accumulation in the mitochondria [48]. The anticancer effect of elesclomol is dependent on its ability to transport extracellular copper ions and the mitochondrial metabolic intensity of cancer cells. With the proposal of cuproptosis as a new form of cell death, elesclomol has also been identified as a cuproptosis inducer [49]. Research has shown that copper ion carriers disrupt redox homeostasis, increase the concentration of redox-active copper, and exploit the ROS susceptibility of cancer cells to induce apoptosis. Buccarelli et al. found that elesclomol specifically transfers extracellular Cu^{2+} into the mitochondria, where FDX1 reduces Cu^{2+} to Cu^{+}, generating a large amount of ROS. This induces oxidative stress in glioblastoma stem-like cells, triggering apoptosis [50].

5. Traditional Chinese Medicine (TCM) Regulation of Cuprotosis

(1) TCM Regulation of Cuprotosis Target Genes

Zhong et al[51] identified 180 Traditional Chinese Medicine (TCM) compounds capable of regulating cuprotosis through a systems pharmacology approach. These TCM compounds are primarily associated with the liver, lung, and spleen meridians, and most are categorized as having cold, warm, bitter, pungent, or sweet properties. This suggests that specific medicinal properties and meridian associations might be linked to their ability to regulate cuprotosis. Further analysis revealed that most cuprotosis targets are regulated by compounds such as vitamin B9, resveratrol, and quercetin. Vitamin B9, a water-soluble vitamin involved in protein and nucleic acid synthesis, regulates 21 cuprotosis-related targets, including FDX1[52]. Resveratrol and quercetin, natural antioxidants and phytoalexins produced by plants in response to injury or pathogen attack, not only exhibit antioxidant, anti-inflammatory, cardioprotective, and anticancer properties but also regulate 15 cuprotosis targets, including FDX1. These compounds demonstrate considerable potential in medical research due to their multifunctional activities. Zhong et al. also identified 278 TCM herbs containing compounds capable of regulating cuprotosis-related proteins. Among them, red dates (Ziziphus jujuba), ginkgo leaves (Ginkgo biloba), and Siberian ginseng (Eleutherococcus senticosus) were found to have significant regulatory effects on cuprotosis proteins and broad pharmacological activities. Clinical trials have demonstrated that red dates are effective in adjunctive treatment for cancer, hypertension, and hypercholesterolemia, while also offering anti-aging, antioxidant, immune-enhancing, and hepatoprotective effects[53]. Compounds in ginkgo leaves, including flavonoids, terpene lactones, phenolic acids, and polysaccharides, have shown antioxidant, anti-inflammatory, neuroprotective, anticancer, and antimicrobial activities[54]. Yun Zhangjun et al[55] constructed a prognostic model for gastric cancer and analyzed the role of cuprotosis-related genes (CRGs) in treatment, revealing that two TCM herbs, spirulina and Cnidium monnieri, have the potential to regulate the FDX1 gene. Spirulina's main component, spirulina polysaccharides, exhibits antioxidant, immune-regulating functions and can significantly inhibit tumor cell proliferation[56]. The active ingredient of Cnidium monnieri, osthole, has demonstrated anti-oxidative stress, antithrombotic, and anticancer effects by targeting the PI3K/AKT pathway and caspase-3-dependent apoptotic pathway to induce tumor cell apoptosis [57][58]. However, no studies have yet confirmed whether spirulina and Cnidium monnieri can induce cancer cell death by directly regulating the FDX1 gene. Therefore, further research is needed to explore their potential role in regulating the mechanisms of cuprotosis. This highlights the significant potential of TCM in targeting key cuprotosis-related genes and pathways, opening avenues for novel therapeutic strategies in cancer treatment.

(2) TCM and Copper Ion Carriers

TCM can enhance the cytotoxic effects on tumor cells by increasing intracellular copper concentrations through copper complexes or copper ion carriers. This elevated copper concentration in tumor cells boosts ROS levels, leading to proteasome inhibition and ultimately inducing apoptosis, thereby exerting toxic effects on cancer cells [59]. Curcumin, a polyphenol extracted from Curcuma longa (turmeric), forms a complex with Cu^{2+}, which further promotes apoptosis in breast cancer cells, inhibiting proliferation and angiogenesis [60]. Yan et al [61] found that the curcumin-copper complex exhibited higher superoxide scavenging activity compared to free curcumin. Moreover, the curcumin-copper complex enhances the activity of antioxidant enzymes and reduces the increase in malondialdehyde (MDA) levels in pheochromocytoma rat neuron cells, demonstrating a protective effect against oxidative stress. This evidence suggests that TCM compounds, when combined with copper ion carriers, can significantly enhance their antitumor properties by increasing ROS production and promoting apoptosis in cancer cells. These copper-based complexes offer a promising approach in cancer therapy, harnessing the unique properties of TCM to target cancer cells more

effectively.

6. Additional Tumor Treatment Measures - Targeted Gene Knockout

In both RNA and protein levels, dihydrolipoamide dehydrogenase (DLD) is part of the complex functional network within tumors. Tumor cells rely on glutaminolysis to fuel the TCA cycle and support the essential biosynthetic processes needed for tumor growth. DLD is a dehydrogenase found in multiple enzyme complexes that regulate energy metabolism. In the TCA cycle, DLD catalyzes the reduction of nicotinamide adenine dinucleotide (NAD^+) to its reduced form, NADH, playing a critical role in tumor biology [62][63]. Qi et al [64] evaluated the expression profile of DLD and found that it is expressed at higher levels in ovarian serous cystadenocarcinoma and endometrial carcinoma compared to normal tissues. In vitro experiments using ovarian serous cystadenocarcinoma cells revealed that DLD mRNA expression levels were significantly higher in cancerous ovarian epithelial cells than in normal cells. When DLD expression was knocked down using DLD-specific siRNA, it inhibited the intracellular NAD+/NADH ratio, which in turn modulated metabolic pathways and suppressed tumor cell proliferation. This study highlights the potential of targeted gene knockout therapies, specifically through DLD silencing, as a promising strategy to disrupt tumor cell metabolism and limit cancer growth. By inhibiting key enzymes like DLD, it is possible to interfere with the metabolic processes that sustain tumor growth, offering a novel approach to cancer treatment

7. Conclusion and Outlook

The use of TCM to induce Cuprotosis and promote tumor cell apoptosis has become a significant topic of interest. Copper, a trace element, plays essential physiological roles in the human body, but at high concentrations, it can be toxic. In tumor cells, copper is involved in processes like oxidative stress, which are closely related to cell survival, proliferation, and apoptosis. Current cancer treatments largely focus on inducing apoptosis in cancer cells, but resistance to apoptosis is a major cause of treatment failure. Cuprotosis, a newly discovered form of cell death linked to mitochondrial respiratory function, has shown great potential as an innovative therapeutic strategy. Imbalances in copper homeostasis not only inhibit angiogenesis in tumor cells but also lead to elevated ROS levels, proteasome dysfunction, and mitochondrial impairment. These alterations suggest that regulating copper metabolism could be a novel target for anticancer therapies. TCM shows unique potential in modulating intracellular copper ion levels, affecting copper uptake, transport, binding, and release, ultimately disrupting copper-involved metabolic pathways and leading to tumor cell apoptosis. Although the key molecular mechanisms of Cuprotosis are becoming clearer, its specific role in different cancers remains incompletely understood. Further high-quality basic research is required to validate the precise relationship between Cuprotosis and tumor progression. Moreover, although some TCM herbs have been identified through network pharmacology as potential regulators of Cuprotosis, these findings have yet to be directly confirmed in laboratory studies. It also remains unclear whether these TCM compounds directly interact with Cuprotosis targets or act upstream or downstream in the biological pathways involved.Future research should focus on exploring the specific modes of action and molecular mechanisms of TCM in regulating Cuprotosis. Detailed experimental studies are needed to confirm the effects of different TCM herbs on tumor cell apoptosis. This will not only enhance our understanding of the pharmacological properties of TCM but also help confirm the efficacy and safety of these treatments, offering new directions and strategies for cancer therapy.

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