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Research Progress of Cuproptosis and Its Role in Osteoporosis

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Abstract: ***Copper is an essential trace element in the human body, participating in various physiological processes. cuproptosis is a new regulatory of cell death, which is caused by the combination of copper ions with proteins in the tricarboxylic acid cycle, leading to the aggregation of Thiocyanate acylated proteins and the down-regulation of iron-sulfur cluster proteins, thus inducing protein toxic stress to cause cell death. It is a new type of death. As an important coenzyme factor, the functional disorder of copper is related to the pathological conditions of osteoporosis. This paper analyzes the between cuproptosis and osteoporosis from the perspective of copper metabolism, the mechanism of cuproptosis and the role of related genes in osteoporosis, providing for the diagnosis, treatment and research of osteoporosis.***

Keywords: Cuproptosis, Copper homeostasis, Osteoporosis, Related genes**.**

1. Introduction

Osteoporosis (OP) is a common systemic disorder of bone metabolism characterised by a decrease in bone density and bone mass, disruption of bone microarchitecture, and increased bone fragility [1]. It is reported that the incidence increases with age, with 40% of osteoporosis patients experiencing a fragility-related fracture during their lifetime [2]. Currently, the primary medications for treating osteoporosis include teriparatide, bisphosphonates, and denosumab. These drugs carry significant side effects for the human body and are costly [3]. Therefore, exploring potential therapeutic targets for osteoporosis holds significant clinical importance.

Copper, as an important coenzyme factor, exhibits functional disruption associated with the pathological conditions of osteoporosis [4]. However, it may be toxic and cause cell death in environments with excessively high cellular concentrations [5]. Excessively high intracellular copper concentrations trigger the aggregation of mitochondrial thiooctanoylated proteins and destabilisation of Fe-S cluster proteins, leading to a novel form of cell death termed Cuproptosis [6]. Mitochondrial autophagy dysfunction accelerates the progression of osteoporosis [7, 8]. Immune infiltration plays a role in diseases such as cancer [9], ulcerative colitis [10], and osteoporosis [11]. Research has confirmed that immune cell infiltration plays a crucial role in the prevention and treatment of copper toxicity [12]. With the emergence of the new term “immune osteoporosis” [13], a novel field concerning the immune microenvironment of osteoporosis has been established.

2. Copper Biology and Copper Homeostasis

Copper is an essential trace element in the human body, participating in various physiological processes including antioxidant defence and neurotransmitter biosynthesis. The ability of copper to transition between two oxidation states is crucial for its catalytic function as a cofactor. Excess copper proves toxic to cells, and intracellular copper levels are tightly regulated by proteins including copper transporters and copper chaperones [14]. A novel form of nutrient sensing and protein regulation—termed ‘metal dissociation’—has emerged, extending copper’s role beyond catalytic proteins to influence cellular processes in both normal physiological states and disease conditions. It functions as a crucial dynamic signalling molecule [15].

The binding sites identified in copper enzymes exhibit marked differences from those found in sensors, chaperones, and transporters responsible for metallic distribution within cells. Copper transporters only transiently bind the metal during the transmembrane permeation process. Cu^+ chaperones play a central role in homeostasis, performing two primary functions: ensuring Cu^+ delivery to apoproteins and preventing Cu^+ from participating in harmful redox reactions as a high-affinity buffer system. Achieving cellular Cu^+ homeostasis requires both inward and outward transporters, with the most characteristic aspect of copper homeostasis being cytoplasmic Cu^+ efflux [16].

3. Mechanisms of Copper-Induced Cell Death and the Discovery of Cuproptosis

Cuproptosis was first proposed by Tsvetkov et al. in 2022. This mechanism differs from known forms of cell death and relies on mitochondrial respiration [17], thereby opening up a new field of research. Copper binding to sulfonylated TCA cycle proteins enhances DLAT’s sulfonylation-dependent oligomerisation and toxic function. Cells sensitive to elesclomol exhibit elevated DLAT oligomer levels, where abnormal sulfonylation-dependent oligomerisation increases toxicity. Excessive copper deposition promotes aggregation of sulphonylated proteins and downregulation of Fe-S cluster proteins, thereby inducing protein toxicity stress leading to cell death. Intracellular copper concentrations are maintained at low levels to prevent accumulation of harmful free intracellular copper [18], and copper ionophore-induced cell death primarily depends on intracellular copper accumulation. The effector mechanisms of cell death [19] involve proteins and lipids, such as apoptosis [20], necroptosis [21], and ferroptosis [22]. Treatment with inhibitors of ferroptosis (ferrostatin-1), necroptosis (necrostatin-1), and oxidative

stress-mediated cell death (N-acetylcysteine) failed to ablate Cuproptosis, indicating a mechanism distinct from known cell death pathways.

4. The Relationship Between Copper Metabolism and Osteoporosis

Copper metabolism involves cellular and systemic uptake, distribution, sequestration, and excretion. To achieve homeostasis and prevent disease, copper metabolism must be strictly regulated [23]. Copper serves as the active centre of numerous biological enzymes in the human body, playing a vital role within the skeletal system. Copper deficiency compromises the mechanical strength of bones due to reduced cross-linking of elastin and collagen, leading to diminished activity of antioxidant enzymes. This further increases osteoclast activation and bone resorption [24]. Copper deficiency may lead to osteoporosis; bone mineral density should be improved by increasing copper intake [25]. Nearly two-thirds of the copper in the human body is stored in muscles and bones. Excretion may be the primary mechanism regulating copper metabolism within the body. This effective interplay between absorption and excretion helps protect the body against both copper toxicity and deficiency [26].

5. The Relationship Between Copper Death Mechanisms and Osteoporosis

Osteoporosis is a condition characterised by a gradual reduction in bone mass over time, leading to an increased incidence of fractures. According to conventional wisdom, the primary factors contributing to osteoporosis comprise a multifactorial imbalance of hormonal changes, deficiencies in calcium and vitamin D, [27] oxidative stress, mitochondrial dysfunction, chronic inflammation, and the normal ageing process. Programmed cell death (PCD), encompassing mechanisms such as apoptosis, autophagy, iron toxicity, heat stress, and necrosis, plays a pivotal role in regulating bone metabolism by influencing osteoblast activity. This suggests that targeting specific regulatory molecules to intervene in PCD may offer an effective strategy for controlling osteoporosis [28]. A recently identified form of controlled cell death induced by excessive copper ions (Cu^{2+}) has been termed Cuproptosis [29]. Copper participates in the tricarboxylic acid cycle within mitochondria, where it exists in the form of cytochrome oxidase (COX) and superoxide dismutase (SOD1). Intracellular copper interacts with numerous mitochondrial proteins and acts upon the lipid acylation component of the tricarboxylic acid (TCA) cycle. Consequently, copper plays a critical role in numerous biological processes, whether in redox homeostasis, iron turnover, cellular oxidative phosphorylation, or cell growth. The form of copper-induced programmed cell death differs markedly from other cell death pathways such as apoptosis, ferrotoxicity, and necrosis. Copper storage diseases can induce cell death, as the accumulation of these copper-binding, lipidylating mitochondrial proteins, coupled with an induced reduction in Fe-S (iron-sulphur) clusters, triggers proteotoxic stress [30]. Significant advances have been made in the field of oxidative metabolism over recent decades. The by-products of energy-generating reactions are primarily reactive oxygen species (ROS), which are predominantly produced within mitochondria. Consequently, mitochondrial dysfunction is regarded as a key factor in the pathogenesis of bone disorders, as the loss of mitochondrial dynamics leads to imbalances in the bone remodelling process. Dysregulation of intracellular copper bioavailability induces oxidative stress and cytotoxicity, rendering copper-mediated death critically important in osteoporosis.

6. The Role of Copper Death-Related Genes in Osteoporosis

Chen et al. [31] employed a genome-wide weighted gene co-expression network analysis algorithm to identify six pivotal genes (MAP2K2, FDX1, COX19, VEGFA, CDKN2A, and NFE2L2) associated with copper death phenotypes. By exploring the potential linkages between these genes in OP and copper death, they propose novel therapeutic and preventive strategies for OP.

6.1 The Role of MAP2K2 in OP

Mitosis-activated protein kinase (MAPK) exerts potent effects in both innate and adaptive immune responses [32]. Cardiofaciocranial syndrome (CFCS) is a rare disorder causing musculoskeletal abnormalities. It arises from heterozygous mutations in the BRAF, MAP2K1, MAP2K2, and KRAS genes, which belong to the RAS/MAPK pathway. The role of RAS signalling in bone homeostasis is well established. MAP2K2 contributes to reduced bone mineral density (BMD) in CFCS patients via the MAPK pathway, thereby impacting skeletal health [33]. The Ras protein expressed in osteogenic progenitor cells governs skeletal development and metabolism. The impaired bone metabolism observed in CFCS arises from the upregulation of the MAPK cascade, the primary signalling pathway regulated by the Ras protein. This suggests alterations in bone mineral density (BMD) may be controlled by this mechanism [34]. Therefore, it plays a pivotal role in the growth and differentiation of osteoblasts, suggesting that MAP2K2 may potentially intervene in therapeutic strategies for osteoporosis patients from the perspective of bone homeostasis.

6.2 The effect of FDX1 on OP

Reports indicate that FDX1 is a key molecule in activating copper ion carriers to induce cell death [35]. FDX1 is recognised as an upstream regulatory molecule for protein sulphhydryl modification, exhibiting reactivity towards copper ion carriers. Recent research indicates that copper ion carriers diminish FDX1-dependent Fe-S cluster biosynthesis [36]. Compared to normal osteoblasts, the expression of FDX1 is significantly elevated at both the mRNA and protein levels in osteosarcoma (OS) cell lines [37]. Interference with the FDX1 gene affects the proliferation and differentiation of osteoblasts [38], suggesting that FDX1 may serve as a potential therapeutic target for osteoporosis by regulating protein levels.

6.3 The effect of COX19 on OP

Non-sensor-mediated mRNA decay (NMD) regulates COX19 in mitochondrial and copper homeostasis [39], NMD precisely regulates COX19 mRNA under varying conditions,

thereby maintaining mitochondrial copper homeostasis. The extended 3′-UTR of COX19 mRNA facilitates NMD-mediated degradation, suggesting that the COX19 3′-UTR may represent a more effective NMD-targeting feature [40]. Research indicates that COX19 is significantly upregulated in osteoporosis, suggesting it may influence the progression of the condition by regulating mitochondrial signalling pathways.

6.4 The Role of VEGFA in OP

Vascular endothelial growth factor (VEGF) is a signalling protein. Reduced VEGF expression diminishes osteoblast differentiation, thereby promoting osteoporosis. The synergistic interaction between genetic variants in the VEGF-A gene and conventional risk factors can predict endothelium-related osteoporosis in postmenopausal women [41]. ERβ plays a crucial role in regulating osteoporosis by promoting VEGFA-mediated osteoblast differentiation [42]. Research indicates that overexpression of GAS5 promotes angiogenesis in osteoblasts by suppressing miR-10a-3p and thereby upregulating VEGFA [43]. This suggests that VEGFA may improve the progression of osteoporosis by promoting vascularisation in osteoblasts.

6.5 The Role of CDKN2A in OP

We have discovered that CDKN2A may be the most significant marker and effector of osteoblast senescence [44]. Increased expression of CDKN2A promotes autophagic activity in somatic cells [45]. Research indicates that osteoblast death is compromised by apoptosis or epigenetic modifications of cyclin-dependent kinase inhibitor 2A (CDKN2A), a key participant in the cell cycle [46]. Therefore, CDKN2A may contribute to the progression of osteoporosis by regulating osteoblast senescence.

6.6 The role of NFE2L2 in OP

NFE2L2 is an alkaline region leucine zipper transcription factor, and its target genes are key factors in resisting oxidative stress [47]. NFE2L2 regulates osteoclastogenesis and bone resorption by modulating ROS production through the control of antioxidant enzymes [48]. NFE2L2 is expressed in osteoblasts and osteoclasts, primarily existing in an inactive form within the cytoplasm. Moderate activation of NFE2L2 plays a crucial role in delaying the progression of osteoporosis [49]. Research indicates that NFE2L2 translocates from the cytoplasm to the nucleus, where it binds to antioxidant response elements (AREs) within genes encoding antioxidant enzymes to mitigate cellular damage from reactive oxygen species (ROS). The NFE2L2 signalling pathway exerts a protective effect in osteoporosis [50]. Therefore, reduced NFE2L2 expression may exacerbate bone loss in patients with osteoporosis.

7. Conclusion

In summary, Copper, acting as a coenzyme factor, can induce copper death when intracellular copper concentrations become excessively high. This process leads to the formation of protein aggregates and destabilisation of Fe-S cluster proteins. Current research indicates copper death is associated with the progression of osteoporosis. Investigating the mechanisms of copper death holds significant potential for influencing osteoporosis management. Furthermore, genes associated with copper death demonstrate developmental potential in osteoporosis, offering novel directions for its prevention and treatment, thereby enhancing current clinical therapeutic approaches. Concurrently, the mechanisms linking copper death to osteoporosis remain incompletely understood. Future research is required to better elucidate copper death pathways, thereby furnishing novel approaches for the prevention and treatment of osteoporosis.

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