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# Cuproptosis—a potential target for the treatment of osteoporosis

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Osteoporosis is an age-related disease of bone metabolism marked by reduced bone mineral density and impaired bone strength. The disease causes the bones to weaken and break more easily. Osteoclasts participate in bone resorption more than osteoblasts participate in bone formation, disrupting bone homeostasis and leading to osteoporosis. Currently, drug therapy for osteoporosis includes calcium supplements, vitamin D, parathyroid hormone, estrogen, calcitonin, bisphosphates, and other medications. These medications are effective in treating osteoporosis but have side effects. Copper is a necessary trace element in the human body, and studies have shown that it links to the development of osteoporosis. Cuproptosis is a recently proposed new type of cell death. Copper-induced cell death regulates by lipoylated components mediated *via* mitochondrial ferredoxin 1; that is, copper binds directly to the lipoylated components of the tricarboxylic acid cycle, resulting in lipoylated protein accumulation and subsequent loss of iron-sulfur cluster proteins, leading to proteotoxic stress and eventually cell death. Therapeutic options for tumor disorders include targeting the intracellular toxicity of copper and cuproptosis. The hypoxic environment in bone and the metabolic pathway of glycolysis to provide energy in cells can inhibit cuproptosis, which may promote the survival and proliferation of various cells, including osteoblasts, osteoclasts, effector T cells, and macrophages, thereby mediating the osteoporosis process. As a result, our group tried to explain the relationship between the role of cuproptosis and its essential regulatory genes, as well as the pathological mechanism of osteoporosis and its effects on various cells. This study intends to investigate a new treatment approach for the clinical treatment of osteoporosis that is beneficial to the treatment of osteoporosis.

## KEYWORDS

osteoporosis, copper, cuproptosis, cuproptosis-related genes, inflammation

## 1 Introduction

The balance of osteoclast-mediated bone resorption and osteoblast-mediated bone formation is directly responsible for maintaining bone homeostasis (1). Disruptions in this balance caused by over-absorption or under-formation result in decreased bone mass and deterioration of bone tissue microarchitecture, resulting in osteoporosis(OP) (1, 2). Osteoporotic fractures usually threaten people's health, especially in older individuals, and impose significant socioeconomic burdens (3). Currently, the most common methods for treating OP include inhibiting osteoclast activity and inducing bone formation (4). Inhibitors that osteoclast differentiation and activation, such as estrogen, bisphosphates, denosumab, calcitonin, and others, have been used in the clinic for many years, but they have numerous side effects (4, 5). Drugs that promote bone formation, such as parathyroid hormone and strontium ranelate, also pose many problems in clinical use, such as nausea, dizziness, and leg cramps with long-term use of the parathyroid hormone (6). The manufacturer recalled Strontium ranelate as early as 2017 because long-term use was toxic to the circulatory system (7). As a result, it is advantageous to investigate new drugs and elucidate novel mechanisms of action in the treatment of OP.

Cell death is linked to OP. For example, ferroptosis disrupts osteoblast differentiation (8), and inducing osteoclast apoptosis can maintain bone mass (9). A new type of cell death, known as "cuproptosis," has recently been proposed (10). In contrast to other recognized death mechanisms, such as pyroptosis and necroptosis, it is a distinct non-apoptotic programmed death mechanism (11). copper initiates it and requires the mediation of protein lipoylation in the mitochondria; specifically, the tricarboxylic acid (TCA) cycle's lipoylated components are directly bound by the copper to cause cuproptosis, resulting in lipoylated protein aggregation and subsequent loss of iron-sulfur cluster protein, which inevitably leads to proteotoxic stress and eventually cell death (10). In addition, using genome-wide CRISPR-Cas9 dysfunction screening, Cuproptosis was linked to 10 essential genes, including regulator Ferredoxin 1(FDX1) and six lipoylated protein genes encoding either lipoic acid pathway elements (three key lipoic acid pathway enzymes - Lipoyltransferase 1(LIPT1), Lipoic Acid Synthetase(LiAS), and Dihydrolipoamide Dehydrogenase(DLD) or lipoylated protein targets (three components of the pyruvate dehydrogenase complex, including Dihydrolipoamide S-acetyltransferase (DLAT), Pyruvate Dehydrogenase E1 Subunit Alpha 1(PDHA1), and Pyruvate Dehydrogenase E1 Subunit Beta(PDHB), and knockdown of the above seven genes could rescue cuproptosis, it also includes the negative regulators Metal Regulatory Transcription Factor 1 (MTF1), Glutaminase(GLS), and Cyclin-Dependent Kinase Inhibitor 2A(CDKN2A) (10). Furthermore, 728 postmenopausal women(aged between 45 and 80 years old) were divided into two groups based on whether they had OP; the bone mineral density and blood copper content of these women were evaluated, and the results showed that the serum copper level of osteoporotic women was lower than that of healthy women (12). There was a statistical

difference between the two groups, and in addition, there was a positive correlation between serum copper level and bone mineral density (12). Similarly, some studies have found that elevated serum copper concentration negatively correlates with the risk of OP (13, 14). Furthermore, some researchers investigated the link between copper intake and OP; the findings revealed that dietary and total copper intake was positively correlated with increased bone mineral density in adults and negatively related to the risk of OP (15). Through the above studies, we found that serum copper levels in patients with OP were low, and total copper intake also affected OP. Therefore, we searched the Web of Science, Pubmed, and other databases for copper, cuproptosis, and cuproptosis-related genes to elucidate the potential link with osteoporosis and explore new therapeutic avenues.

## 2 The potential link between copper and OP

Our bodies contain between 50 and 120 mg of copper, with muscles and bones accounting for roughly two-thirds of the copper content (16, 17). Copper is essential for energy metabolism at the cellular level because its primary function is to constitute the enzyme that transfers electrons (oxidase) to reduce molecular oxygen (18). Among these enzymes, we discovered lysyl oxidase, a copper-dependent monoamine oxidase that can use lysine and hydroxylysine (found in collagen and elastin) as substrates to produce cross-links required for the development of connective tissues such as bones (19–21). In addition, copper is a cofactor for many enzymes in collagen synthesis, especially metalloenzymes (22). Because it constitutes an essential element, its deficiency may lead to bone metabolism disorders and contribute to OP development (23, 24). Several *in vitro* studies have found that copper has a positive effect on the regulation of bone metabolism cells, and copper has been found to stimulate the differentiation of mesenchymal stem cells into the osteogenic lineage (25). Furthermore, copper is a cofactor of antioxidant enzymes that scavenge bone radicals and promote osteoblast activity (26). However, it has been noted that the beneficial effects of copper are dose-dependent, with low doses promoting osteoblast growth and high doses causing cytotoxicity (27). Furthermore, Li et al. discovered that copper deficiency might reduce superoxide dismutase activity in the antioxidant enzyme system, resulting in osteoclast activation and increased bone resorption (18). Therefore, the trace element copper appears to be linked to OP, which intrigued our interest in the concept of "cuproptosis," recently proposed by Tsvetkov et al. (10).

## 3 The potential link between cuproptosis and OP

Tsvetkov et al. provided a detailed description of cuproptosis (10). First, copper ionophore-induced cell death primarily depends on intracellular copper accumulation (10). Second, copper

ionophores cause a distinct type of regulated cell death that differs from other known death mechanisms (10). Furthermore, cells that rely on mitochondrial respiration are nearly 1000-fold more sensitive to copper ionophore-induced cell death than cells that rely on glycolysis; however, mitochondrial respiration can be inhibited under various conditions, including hypoxia, mitochondrial antioxidants, inhibitors of mitochondrial function, and the presence of fatty acids, in addition, glutathione depletion can lead to copper-dependent cell death (10). oligomers' accumulation is also critical for the development of cuproptosis (10). Finally, the knockdown of 7 genes (including "FDX1, LIPT1, LIAS, and DLD, as well as DLAT, PDHA1, and PDHB") rescued copper-induced cell death (10). According to research on the epidemiology and pathogenesis of OP, the associated elevated ROS can cause OP (28). Studies have shown that hypoxia is critical in protecting bones from ROS-mediated damage (29), while bones and bone marrow cavities are in natural hypoxia. Recently, it has been shown that osteoblasts and osteoclasts, which maintain bone homeostasis, are oxygen-sensing cells (30), and hypoxia regulates bone remodeling and bone remodeling turnover (31). However, continuous exposure to hypoxic conditions will increase (NF)-kB-ligand(RANKL) formation, promoting osteoclast formation (32). OP occurs due to changes in the bone marrow microenvironment and dysregulation of homeostasis among cells. The metabolic mechanisms associated with cuproptosis may be related to these cells (33). For example, glutamine plays an essential role in the energy metabolism of osteoblasts (34). Biltz RM et al. early found active glutamine absorption and metabolism in explants of the calvaria and long bones (34). Recent studies have shown that matrix mineralization in calvarial osteoblast cultures requires glutamine (35). Stable isotope tracer experiments provide that converting glutamine into citrate thus contributes to the production of energy in the mitochondria of osteoblast precursors (36). Glutamine is also associated with cuproptosis, and a reduction in its level significantly inhibits cuproptosis. Therefore, it may affect the energy metabolism of osteoblasts. Hypoxia may also inhibit cuproptosis (10), but the relationship between hypoxia and copper is very complex. Hypoxia can inhibit the antioxidant defense mechanism by increasing ROS, thus promoting the cytotoxicity of copper (37). Many studies have found that ROS-induced oxidative stress plays a vital role in OP. ROS can indirectly affect osteoclast differentiation, survival, and activation by stimulating bone formation-associated cells to produce osteoprotegerin, macrophage colony-stimulating factor (M-CSF), and RANKL, which are important regulatory factors that recognize osteoclast precursor cells and osteoclasts to conduct bone resorption signals. In addition, effector T cells function through an mTOR-dependent pathway, using glycolytic uptake of glutamine and glucose for energy, which may also inhibit cuproptosis (33). In recent years, some people have studied the relationship between the occurrence and development of OP and T cells. In addition, M1 pro-inflammatory macrophages are glycolytic cells that can inhibit cuproptosis while releasing various pro-inflammatory factors involved in the development and progression of OP. These factors may link OP to cuproptosis

(Table 1). In addition, a possible correlation between crucial genes related to cuproptosis and OP is described (Figure 1).

### 3.1 The potential link between PDHA1 and OP

PDHA1 may be associated with OP through glycolysis. PDHA1 is a pyruvate dehydrogenase complex(PDC) member, which is essential in glucose metabolism and involves mitochondrial oxidative phosphorylation and the TCA cycle (38). Inhibition of PDHA1 can promote the glycolysis of tumors by down-regulating the activity of PDC (39), increasing glucose and glutamine consumption, and inhibiting oxidative phosphorylation (40). During oxidative phosphorylation, the PDC converts pyruvate to acetyl coenzyme A (33). PDHA1 is a crucial element that connects glycolysis and the TCA cycle (41). Glycolysis converts glucose to pyruvate, which is further converted to acetyl coenzyme A or lactic acid fermentation, depending on aerobic or anaerobic conditions (42). Glucose has long been considered the primary nutrient for osteoblasts; Borle AB et al. showed that bone explants and primary cultured calvaria osteoblasts consume glucose rapidly (43). Zoch ML et al. confirmed the notable glucose absorption by mouse bone (44). Glut transporters are primarily responsible for glucose uptake in osteoblast lineage cells. One study showed that Glut1 and Glut3 were detected in osteoblast cell lines (45). Lactate is the principal end product of glucose metabolism in osteoblasts, regardless of oxygen conditions (46). Historical studies of bone slices or primary calvaria osteoblasts have reported that most glucose carbon is secreted in lactate, even in large amounts of oxygen (47). The production of lactate from glucose under conditions of sufficient oxygen is similar to the Warburg effect observed in many cancer cells, also known as aerobic glycolysis (48). In line with this study, Guntur et al. demonstrated that aerobic glycolysis is the main mode of glucose metabolism in primary calvarial osteoblasts (49). Notably, stimulation of aerobic glycolysis by stabilizing Hifa in preosteoblasts increased osteoblast production and bone formation in mice. Therefore, it can be confirmed that glycolysis is the primary metabolic pathway to meet the needs of ATP during osteoblast differentiation. Transcription factor RUNX family transcription factor 2 (RUNX2) can promote osteoblast differentiation and plays an essential role in bone development and metabolism. In addition, RUNX2 can promote the proliferation of tumor cells by inhibiting the expression of PDHA1 and mitochondrial oxygen consumption rate (50). The glucose metabolism in osteoblasts is similar to that in tumor cells, both of which promote cell proliferation indirectly or directly by enhancing the glycolysis pathway. Therefore, we speculate that PDHA1 may be involved in the proliferation and differentiation of osteoblasts by interfering with the process of glycolysis, and PDHA1 in osteoblasts maybe is suppressed, leading to the glycolysis and proliferation of osteoblasts to affect the course of OP patients directly.

PDHA1 may be associated with OP by regulating inflammation. The NLR Family Pyrin Domain Containing 3(NLRP3) inflammasome was first reported and studied by Martinon and

TABLE 1 The direct or indirect link between cuproptosis-related genes and OP.

Genes related to cuproptosis	The bridge between genes and OP	The relationship between genes and OP
PDHA1	Osteoblasts, osteoclasts, macrophages	PDHA1 inhibition may contribute to osteoblast proliferation; PDHA1 activation affects osteoblasts and macrophages by inhibiting inflammatory factor release.
PDHB	Treg cells, macrophages, osteoblasts	DJ-1 binds to PDHB in Tregs to maintain Treg cell differentiation and T cell integrity. PDHB may affect macrophages by inhibiting ERK signaling; PDHB may affect osteoblast differentiation by affecting mitochondrial homeostasis.
GLS	BMSCs, osteoblasts, osteoclasts, osteocytes, chondrocytes, macrophages	GLS1 affects cells by promoting glutamine metabolism, redox homeostasis, efferocytosis, and antioxidant function.
LIAS	Treg cells, CD4+T cells, macrophages	LIAS is mainly associated with oxidative stress and inflammation.
DLAT	osteoclasts	DLAT may affect OP mainly by influencing the conversion of pyruvate to acetyl-CoA in PDC and mitochondrial metabolism.
FDX1	Macrophages, Treg cells, CD4+T cells	FDX1 may affect cells through metabolic, immune-related pathways.
CDKN2A	Bone marrow adipocytes, BMSCs, osteoblasts, osteoclasts, macrophages, etc.	The P16(INK4A) protein encoded by CDKN2A is a typical marker of cellular senescence- release of senescent cells and inflammatory factors involved in the progression of osteoporosis.
DLD	Osteoclasts, osteoblasts	DLD is associated with osteoporosis through antioxidation and affects energy production in cell metabolism. In addition, DLD can also participate in the treatment of osteoporosis by promoting osseointegration.
MTF1	Osteoblasts, osteoclasts, macrophages, etc.	MTF1 has a direct or indirect relationship with osteoporosis by controlling the balance of metal ions and redox, regulating the expression of related genes, and protecting against metal toxicity.
LIPT1	Osteoblasts, osteoclasts, macrophages, etc.	LIPT1 may be involved in osteoporosis by participating in the tricarboxylic acid cycle and affecting mitochondrial energy metabolism.

his team in 2002, and it is a multiprotein complex that can respond to physiological and pathogenic stimuli in the body (51). A recent study reported that the NLRP3 inflammasome plays a crucial role in bone resorption (52); and plays a vital role in OP (53, 54). Lin et al. found that when cells were stimulated with NLRP3 inflammasome agonists, lactate fermentation was activated to produce lactate,

which induced PKR phosphorylation, activating NLRP3 inflammasomes; in short, activation of NLRP3 inflammasomes requires fermentation of lactic acid (42). Inhibition of PDHA1 will damage pyruvate conversion to acetyl coenzyme A (33). In addition, NLRP3 inflammasomes play a crucial role in host defense by promoting caspase I activation and maturation of IL-1β and IL-

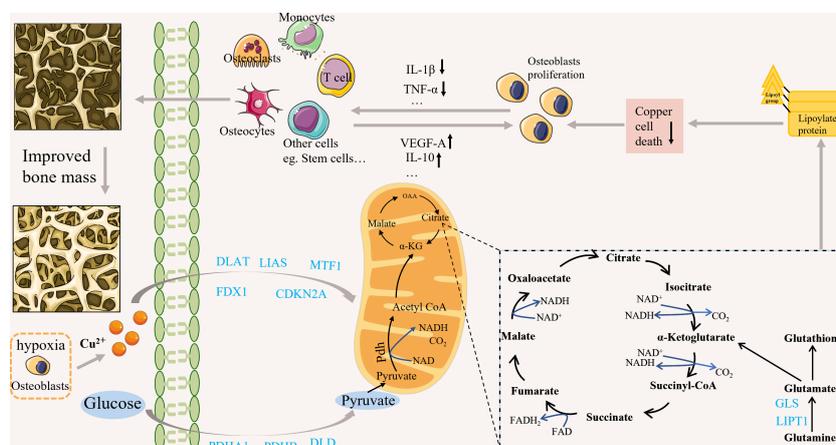


FIGURE 1 Possible correlation between crucial genes related to cuproptosis and OP. Copper can induce cuproptosis by affecting the TCA cycle. Cuproptosis may be associated with osteoporosis by affecting cell function; for example, it may alleviate osteoporosis by affecting the secretion of cellular inflammatory and angiogenic factors. cuproptosis-related genes may be associated with osteoporosis through various mechanisms, but the specific mechanisms need further study.

18, and dysregulation of their activity leads to auto-inflammatory diseases (55). OP is a chronic inflammatory disease. Macrophages are one of the most critical antigen presenters in the body and are an essential tool for the body to remove pathogenic microorganisms. Osteoclasts are differentiated from mononuclear macrophages, highly differentiated multinucleated giant cells directly involved in bone resorption. Macrophages, the precursor cells of osteoclasts, contain receptor activator of nuclear factor- $\kappa$ B (RANK), which can be induced to form osteoclasts *via* M-CSF and receptor activator of RANKL to participate in bone resorption (2). Scholars now generally agree that macrophages in the local microenvironment are mainly divided into classic (M1) macrophages and atypical (M2) macrophages (56). M1-type macrophages mainly play a pro-inflammatory, antibacterial, and antigen-presenting function, while M2-type macrophages mainly play an inhibitory role in inflammation and tissue repair (57). Recently, Cui et al. found that bionic nanovesicles transforming skeletal endothelial cell-associated secretory phenotypes can treat OP due to suppressed M1 macrophage polarization and enhanced M2 polarization (58). Wei Tong et al. found that deacetylation of macrophage SIRT-3 at lysine 83 can activate PDHA1 and inhibit the activation of NLRP3 inflammasome and the release of IL-1 $\beta$  (59). In conclusion, PDHA1 may be an effective regulator of glycolysis and inflammation and is regulated by distinct transcriptional mechanisms (33).

### 3.2 The potential link between PDHB and OP

PDHB, like PDHA1, belongs to two E1 subtypes of the pyruvate dehydrogenase complex, located mainly in the mitochondria of cells, which catalyze the conversion of glucose-derived pyruvate to acetyl coenzyme A (60). It is the critical component linking glycolysis and TCA cycle metabolic pathways. Mutations or defects in the PDHB gene can lead to various metabolic diseases (61, 62). On the other hand, Acetyl coenzyme A promotes the expression of inflammatory cytokines by regulating histone acetylation (63). Danileviciute et al. identified deglycase DJ-1 (encoded by the PARK7 gene, a critical familial Parkinson's disease gene) as a pacemaker regulating pyruvate dehydrogenase (PDH) activity in CD4 regulatory T cells; Deglycase DJ-1 binds to PDHB in Tregs and inhibits the phosphorylation of PDHA, thereby promoting PDH activity and oxidative phosphorylation to keep T cells functional integrity and the differentiation of Treg cells (64). An extracellular signal-regulated kinase (ERK) is a critical member of the mitogen-activated protein kinase MAPKs family, which plays an essential role in the development of OP; Wang et al. found that isobavachalcone can prevent OP by inhibiting the ERK activation, NF- $\kappa$ B pathways, and polarization of M1 macrophage (65). Recently, a study on nasopharyngeal carcinoma showed that PDHB could inhibit RasV12-driven ERK signal transduction and cell growth (66). PDHB is located in mitochondria, and the mutual effect between PDHB and NIMA-associated kinase ten may be indispensable to maintaining mitochondrial function; the knockdown of NIMA-associated kinase ten will damage the respiration of mitochondria (67). There are more mitochondria

per unit surface area of osteoclasts than almost any other cell, and the energy needed for osteoclast differentiation mainly comes from mitochondrial oxidative metabolism (68). Therefore, PDHB may affect osteoclast differentiation through interaction with NIMA-associated kinase ten.

### 3.3 The potential link between GLS and OP

Glutamine is the most abundant non-essential amino acid in circulation and has a variety of metabolic uses in cells (69). Glutamine metabolism has emerged as a critical regulator of many cellular processes in diverse pathologies (70). It is initiated by GLS, the primary enzyme responsible for glutamine catabolism (70). The activity of GLS is encoded by two protein isoforms, Kidney type Glutaminase A (KGA, or glutaminase 1, encoded by Gls1), and Liver type Glutaminase (LGA, or glutaminase 2, encoded by Gls2) (70). Huang et al. used quantitative PCR (qPCR) analysis to show that bone marrow mesenchymal stromal cells (BMSCs) express Gls1 at much higher levels than Gls2, Gls2 expression is even negligible under both undifferentiated and differentiated conditions, suggesting that Gls1 encodes most of the GLS activity in BMSCs (70). That Gls1 has a regulatory BMSCs proliferation and osteoblast endowment (70). The mechanism may be because inhibition of GLS significantly reduces the content of downstream metabolite  $\alpha$ -ketoglutarate ( $\alpha$ -KG), and transaminase-dependent  $\alpha$ -KG production is critical for the proliferation, specification, and differentiation of BMSCs (70). In addition, it has been found that micRO-RNA-200a-3p is highly expressed in the serum of OP patients; it can inhibit osteogenic differentiation of BMSCs by targeting GLS, thus accelerating the progression of OP (71). Knockdown of micRO-RNA-200a-3p can promote osteogenic differentiation of BMSCs; GLS overexpression similarly reversed the inhibitory effect of micRO-RNA-200a-3p on osteogenic differentiation (71). miR-206 can directly bind to the 3'-UTR region of GLS mRNA, thereby inhibiting GLS expression and glutamine metabolism; rescue experiments to restore GLS lead to recovery of glutamine metabolism and osteogenic differentiation (72). GLS expression and activity are regulated by Wnt/Mtorc1 signaling during osteoblast differentiation (36). MTOR, a protein synthesis master regulator, affects the transcriptional activity of ERR  $\alpha$  (73). Glutaminase (GLS) is the target gene of estrogen-related receptor  $\alpha$  (ERR  $\alpha$ ) (73). ERR  $\alpha$  and its coactivator PGC-1  $\alpha$  direct GLS, especially in the early stage of osteogenic differentiation, guide mitochondrial glutamine-dependent anaplerosis and make  $\alpha$ -KG production into the TCA cycle (73). In conclusion, mTOR affects the ERR $\alpha$ /PGC-1 $\alpha$ /Gls signaling pathway to stimulate mitochondrial glutamine anaplerosis, coordinating energy production to help meet increased energy demand from new protein synthesis and promoting osteogenic differentiation of BMSCs (73). GLS inhibition impairs the induction of osteoblast markers (74). In contrast, Huang et al. found that the ERR $\alpha$ /PGC-1 $\beta$ /Gls regulation axis can drive metabolic adaptation that promotes osteoblast differentiation (75). The effect on osteoblasts or osteoclasts may depend on the coactivator of ERR  $\alpha$ , namely PGC-1 $\alpha$  or PGC-1 $\beta$ . Hypoxia-inducible factor HIF-1 $\alpha$  (a key

transcription factor in hypoxia signaling) mediates glutathione synthesis through stable stimulation of GLS, allowing cells to maintain redox homeostasis even during baseline, oxidative or nutritional stress to promote adaptive cellular metabolism, which in turn supports the survival of implanted osteoblasts to promote bone regeneration (76). Like this study, Steve et al. found that GLS1-mediated glutamine catabolism supports anabolic processes and redox homeostasis in osteoprogenitors, thereby promoting the proliferation and differentiation of osteoprogenitors into osteoblasts, at the same time, the deletion of GLS1 leads to an osteoporotic phenotype by impairing bone formation (77). The growth and development of longitudinal bones are through endochondral ossification in the growth plate, during which chondrocytes undergo proliferation, hypertrophy, apoptosis, and eventual replacement by osteoblasts (78). Yue et al. obtained abundant low molecular weight peptides of glutamate/glutamine from sea cucumber intestine by enzymatic hydrolysis and administered orally to adolescent mice and found that sea cucumber intestine promoted the Sox9 entry into the chondrogenic gene enhancer region to accelerate the cell cycle through upregulation of glutamine-mediated histone acetylation and ultimately accelerated growth plate chondrocyte proliferation, effectively promoting longitudinal bone growth, in contrast, deletion of GLS1 would inhibit glutamine metabolism in growth plate chondrocytes and interfere with normal chondrocyte function, ultimately leading to stunted long bone growth (79).

Macrophage reprogramming and phenotypic polarization are controlled by glutaminolysis, an essential metabolic factor (80). Some investigations have revealed that  $\alpha$ -KG derives from glutaminolysis is necessary for M2 polarization (81–83). Several regulatory mechanisms have been identified for the involvement of glutamine in macrophage activation (84). P-C H et al. found that  $\alpha$ -KG produced by glutaminolysis facilitates M2 phenotype formation through epigenetic reprogramming of M2-specific marker genes mediated by histone demethylase Jmjd3 (81). M-N A et al. have shown that UDP-GlcNAc is essential for the polarization of M2 macrophages because it is responsible for the M2 marker proteins glycosylation, and more than one-half of the nitrogen in UDP-GlcNAc comes from glutamine (85). Feng et al. found that GLS1-mediated glutaminolysis promoted the proliferation and adhesion of mouse BMDMs and the infiltration and activation of M2 macrophages, and promoted the secretion of pro-angiogenic cytokines such as VEGF-A (84). The weakening of the ability of macrophages to clear apoptotic cells (i.e., efferocytosis) will enhance the bone resorption capacity of osteoclasts, which is another crucial factor in OP. Merlin et al. revealed that GLS1-mediated glutaminolysis is essential to promote the clearance of apoptotic cells (i.e., efferocytosis) by macrophages during homeostasis in mice (86). The mechanism involves non-classical glutamine metabolism; thus, it plays a massive role in removing dying cells and maintaining tissue homeostasis (86). There is a link between OP and inflammation *in vivo*; in other words, OP is also known as a chronic inflammatory disease. ROS are the product of oxygen metabolism, and high concentrations of ROS will disrupt the balance of oxidants and antioxidants, leading to inflammatory diseases. NF- $\kappa$ B is another critical factor regulating inflammation.

Glutamine metabolism is also significant during inflammation, as energy, biosynthesis, and antioxidant capacity are essential for an appropriate immune response (87). Of course, because glutaminase plays an essential role in metabolism and antioxidant function, its transcription is also tightly regulated (88, 89). Merlin et al. found that GLS1 overexpression can limit ROS production (86). In a rat liver transplantation model, DM- $\alpha$ KG (a cell-permeable analog of  $\alpha$ -KG) perfusion inhibited NF- $\kappa$ B activity, up-regulated p-GSK3 $\beta$  and Suppressor Of Cytokine Signaling 1(SOCS1) expression in Kupffer cells, shifted M1/M2 balance toward an anti-inflammatory direction, and inhibited serum secretion of pro-inflammatory cytokines and increased IL-10 (90). We also found that some studies have shown that cytokines that promote OP can up-regulate the expression of GLS1. For example, STAT1 is one of the candidate genes for human OP (91). It has been reported that INF- $\alpha$  in monocyte-derived macrophages (MDMs) can increase human GLS1 promoter activity through STAT1 phosphorylation, thereby enhancing GLS1 expression and glutamate production (92, 93). In addition, IL-1 $\beta$  and TNF- $\alpha$  can up-regulate GLS1 expression, which induces glutamate production leading to neurotoxicity (94). In conclusion, GLS1 affects multiple cells mainly through glutamine metabolism and antioxidant function, thus influencing the course of OP.

### 3.4 The potential link between LIAS and OP

LIAS is a Fe-S cluster protein that catalyzes the final step in lipoic acid biosynthesis (95). Lipoic acid(LA) is an essential metabolite in various biochemical actions, such as antioxidants. It performs various cellular functions in various metabolic pathways (95), involving the decarboxylation reactions of pyruvate and  $\alpha$ -KG (96, 97). It plays a vital role in mitochondrial energy metabolism (98). Mitochondria are known to be the “energy factory” of the cell, providing energy through oxidative phosphorylation and ATP synthesis. In addition, mitochondria are the primary source of ROS and the most direct target of ROS (99). Oxidative stress occurs when there is an imbalance between the production of ROS and the scavenging capacity of the cellular antioxidant system, and it is thought to be a pathogenic factor in many disease states, including OP (100). LIAS is mainly related to oxidative stress and inflammation. LIAS overexpression decreases inflammatory responses (including reduced expression of pro-inflammatory cytokines/chemokines and inhibition of NF- $\kappa$ B activity) as well as reduces oxidative stress and enhances antioxidant defenses (including increased production of NRF2 and Lias) to potentially protect mitochondrial function in diabetic nephropathy mice (101). Similar to this study, Zhao et al. found that silica significantly increased oxidative stress in wild-type and Lias<sup>H/H</sup> mice (LIAS overexpression in mice) (102). However, unlike wild-type mice, Lias<sup>H/H</sup> mice reduced oxidative stress induced by silica through NRF2 signaling; they downregulated pro-inflammatory cytokine expression by inhibiting NF- $\kappa$ B activation, alleviating the severity of significant pathological alterations in the early stages of silica-induced pulmonary fibrosis (102). Overexpression of LIAS in experimental atherosclerotic mice significantly increased the

number of Tregs (which can produce anti-inflammatory cytokines such as IL-10); it was accompanied by a decrease in the infiltration of CD4<sup>+</sup> T cells (which can produce pro-inflammatory cytokines such as INF- $\gamma$ ) to reduce atherosclerosis (103). Corresponding to this study, as early as 2010, Yi et al. found that genetic reduction of LIAS expression increased atherosclerosis in male apolipoprotein E deficient mice, which was associated with a reduction in endogenous antioxidant defense reservoir due to reduced LIAS expression (104). When LIAS is mutated, it stabilizes HIF-1 $\alpha$  in a non-hydroxylated form and leads to HIF-1 activation by inhibiting prolyl hydroxylases (PHDs) activity, which may lead to enhanced glycolytic effects in cells (105). In addition, LIAS's expression level was reported to be negatively correlated with M1 macrophages (106). The above results suggest that LIAS may be included as a new marker in the research field for OP treatment.

### 3.5 The potential link between DLAT and OP

DLAT is the E2 subunit of the PDC in the glucose catabolism pathway, and it plays a critical catalytic role in converting pyruvate to acetyl-CoA (107). E4 transcription factor 1 is a widely expressed transcriptional regulator that plays a crucial role in cell cycle control and proliferation (108); it can interact with OP *via* P53 (109, 110). E4 transcription factor 1 regulates DLAT, and these two ingredients maybe regulate OP together (111). SIRT2 is a kind of deacetylase with a wide range of physiological functions, and Jing et al. found that SIRT2 deficiency prevents age-related bone loss in rats by inhibiting osteoclast production and that DLAT, a substrate of SIRT2, may be involved in regulating the process of OP (112, 113). In addition, component 1Q subcomponent-binding protein in the mitochondria induces activation of the NLRP3 inflammasome (114). It can regulate mitochondrial metabolism by binding to DLAT and affecting the activity of PDH (115). Thus, DLAT may affect OP mainly by influencing the conversion of pyruvate to acetyl-CoA in PDC and mitochondrial metabolism.

### 3.6 The potential link between FDX1 and OP

FDX1, also known as adrenodoxin or heptaredoxin, is a subunit of the augmin complex and is present in the mitochondrial matrix (116, 117). It encodes a small iron-sulfur protein with low redox potential and molecular weight and contains an iron-sulfur cluster (118). FDX1 transfers electrons from NADPH to mitochondrial cytochrome P450 through the ferredoxin reductase and participates in the metabolism of steroids, vitamin D, and bile acids (119, 120). In addition, FDX1 affects immune cells (e.g., monocytes, M1/M2 macrophages, dendritic cells, neutrophils, mast cells, B cells, regulatory T cells, CD4<sup>+</sup> T cells, NK cells, Etc.) and immune-related genes (e.g., CXCL16, CD40, TNFRSF15, 25, CTLA4, CD274, PDCD1, NRP1, Etc.) (116, 117, 121–123). FDX1 was significantly associated with DNA and RNA methylation (e.g., m6A) (121). The FDX1 gene may promote ATP production, and in

addition, it is closely associated with the metabolism of glucose, fatty acids, and amino acids (124). FDX1 co-expressed genes are mainly involved in various metabolic processes, such as mitochondrial gene expression and mitochondrial respiratory chain complex assembly, and are associated with the Notch signaling pathway (117). Zhang Zhen et al. used LASSO Cox regression models to construct a novel CRRS (cuproptosis-related risk score) based on FDX1 and its related genes (125). They found that patients in the high CRRS group exhibited cancer-related pathways such as WNT signaling, Notch pathway, and Hedgehog signaling, while many genes encoding glycolytic pathways (e.g., hexokinase 2) were significantly upregulated in hepatocellular carcinoma patients with high CRRS (125). In addition, forkhead box M1, a known transcription factor promoting glycolysis in some cancers by binding to the promoters of glycolytic enzyme genes, including glucose transporters (GLUTs), also increased in patients in the high CRRS group (125). Hypoxia-inducible factor-1 (HIF-1) determines glucose consumption through oxidation or glycolysis, and HIF1A is also elevated in patients in the high-CRRS group (125). In addition, the genes with the highest mutation frequencies in the low CRRS and high CRRS groups included catenin beta one and tumor protein P53 (125). CRRS also showed significant positive correlations with IFN $\gamma$  markers (IFNG and STAT1), intercellular adhesion molecule 1, and colony-stimulating factor 1 receptor (125). Similar to some of the results of Zhang Zhen et al. is the study by Zhang Chi et al., who found that FDX1 was associated with immune-related pathways such as inflammatory response, IFN $\gamma$  response, TNF- $\alpha$ /NF-KB signaling pathway (123).

All the genes, cells, or signaling pathways mentioned above are osteoporosis-related. Therefore, FDX1 may be involved in OP through metabolic, immune cells, immune-related genes, and inflammatory pathways.

### 3.7 The potential link between CDKN2A and OP

CDKN2A gene is adjacent to the 9P21.3 genomic region and is a key cell cycle regulator (126). It encodes the P16 (INK4A) protein, a typical marker of cellular senescence (127). The gerontology community increasingly recognizes OP risk factors, including aging (128, 129). Aging reduces the defense against oxidative stress, aggravates the loss of sex steroids, and adversely affects bones (130). Skeletal aging is accompanied by changes in the tissue microenvironment with increased levels of pro-inflammatory cytokines (130), a key factor contributing to OP. Cellular senescence is a basic aging mechanism that can lead to various age-related diseases (131). Cellular senescence has become one of the signs of aging and a major contributor to age-related diseases, including OP (132). Cellular senescence is characterized by growth inhibition, functional alterations, and the presence of so-called senescence-associated secretory phenotypes (SASP), including the expression of inflammatory and trophic factors and tissue remodeling matrix metalloproteinases (MMP) (133). p16 controls the G1 phase of the cell cycle, a key regulator of cellular senescence frequently inactivated in cancer; it is also thought to be

associated with many age-related pathologies, including OP (134). The cell cycle-dependent kinase inhibitor p16 is not only a recognized indicator of cellular senescence, but it also acts as a key effector of cellular senescence (127). The expression level of p16 gradually increases during the development of physiological aging and aging-related diseases (135). Senescent cells in the bone microenvironment increase with aging, producing a pro-inflammatory secretion that leads to increased bone resorption and reduced bone formation; methods that eliminate senescent cells or impair their pro-inflammatory secretion production have been shown to prevent age-related bone loss in mice (136). Targeted removal of p16<sup>Ink4a</sup>-expressing cells in p16-INK-ATTAC (p16<sup>Ink4a</sup> apoptosis through targeted activation of caspases) mice have been successfully used to understand the effects of removal of p16<sup>Ink4a</sup>-expressing senescent cells in a variety of age-related diseases, including OP (137, 138). Early observations using the INK-ATTAC model by Farr et al. showed that p16-overexpressing cells in old mice increase the number of osteoclasts (138). p16<sup>Ink4a</sup> directly interacts with and activates the major adipogenic regulator peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) (139). Eliminating p16-expressing cells in aged INK-ATTAC mice reduces the number of bone marrow adipocytes (138). Similarly, eliminating p16-expressing cells in aged p16-3MR mice (an alternative to the INK-ATTAC model of p16-expressing cell elimination) reduces the adipogenic potential of bone marrow cells (140). The p16-3MR transgenic mouse eliminates senescent osteoclast progenitors but does not eliminate senescent osteocytes (140). However, Farr et al. showed that INK-ATTAC transgenic mice effectively reduced the number of senescent osteocytes in old mice (138). However, it is still unknown whether the elimination of senescent osteocytes themselves is the cause of the increase in bone mass seen in the INK-ATTAC model (140). Yang et al. recently demonstrated that 1,25(OH)<sub>2</sub>D<sub>3</sub> plays a role in the prevention of age-related osteoporosis by up-regulating Ezh2 through vitamin D receptor-mediated transcription, increasing H3K27me3 and repressing p16 transcription, thus promoting the proliferation and osteogenesis of BMMSCs and inhibiting their senescence, while also stimulating bone formation in osteoblasts and inhibiting osteocyte senescence, SASP, and osteoclastic bone resorption (141). Bmi1 overexpression in MSCs can stimulate MSC proliferation and differentiation into osteoblasts by inactivating p16/p19 signaling and inhibiting oxidative stress, thereby enhancing osteoblast bone formation and exerting anti-osteoporosis effects (142). In addition, c-Abi (a non-receptor tyrosine kinase) regulates BMP-Smad1/5/8 and BMP-Tak1-Mek1/2-Erk1/2 pathways by phosphorylating BMP receptor IA (BMPRIA) to control p16<sup>Ink4a</sup> expression and after that osteoblast expansion and bone formation (143). P16 is the direct target of HIF-2  $\alpha$  in osteoclasts, which mediates the enhancement of bone resorption activity of senescence-related osteoclasts (144). In addition, p16<sup>Ink4a</sup> can regulate the activation and polarization of macrophages (i.e., BMDMs) via the JAK2-STAT1 pathway. Its deletion regulates the phenotype of macrophages exhibiting a phenotype similar to IL-4-induced macrophage polarization (i.e., M2 type), resulting in a significant decrease in the expression of inflammatory genes (e.g., IL-6 and TNF) (145). The expression of

p16<sup>Ink4a</sup> is also significantly higher with aging in B cells, T cells, myeloid cells, and osteoblast progenitor cells, leading to bone loss (130). CDKN2A has been linked to OP through the P16 protein it encodes, primarily involved in cellular senescence.

### 3.8 The potential link between DLD and OP

DLD is a member of the redox family of Flavin proteins (146). It can participate in catalysis because the protein consists of 474 amino acids, including an active disulfide bridge and a FAD cofactor, which endow it with activity (147). The normal function of PDC requires the participation of DLD (148). By regulating the components of pyruvate, PDC converts it into acetyl-CoA and guides acetyl-CoA into the Krebs cycle to produce CO<sub>2</sub>, NADH, and H<sup>+</sup>; PDC plays an essential role in this process (147). The primary function of DLD is to promote the oxidation of lipoyl molecules by reducing NAD<sup>+</sup> to NADH and to prepare for the subsequent cyclic catalytic reaction of lipoyl molecules (147). Therefore, DLD is vital in maintaining cell energy metabolism and the tricarboxylic acid cycle (149). DLD has been proven to have antioxidant properties in humans because it contains important cofactors FAD and NADH (149). In addition,  $\alpha$ -lipoic acid is the substrate of DLD, which can treat various chronic diseases, including OP (150, 151). Glucose plays a crucial role in bone metabolism and plays a key role in maintaining the homeostasis between bone formation and bone resorption (152). The enzyme encoded by DLD is part of the PDH enzyme complex, which directly promotes the energy metabolism of glucose (153). Inhibiting the activity of the DLD enzyme decreased the glycolysis catabolism of glucose (153). Glycolysis is an important energy-driven pathway in the process of osteoclasts differentiation (154). Therefore, increasing the process of glycolysis is essential for osteoclasts formation (155), and inhibition of the DLD enzyme may inhibit osteoclasts formation. In addition, it has been found that DLD<sup>RGD</sup> can be prepared by attaching RGD molecules to the N-terminal and C-terminal of DLD; This conjugate can be coupled with TiO<sub>2</sub> coated with the implant and promote the osseointegration of osteoblasts and implants through coupling with specific integrins on the surface of bone-forming cells (156–158). Therefore, DLD<sup>RGD</sup> may be helpful in the treatment of a variety of orthopedic diseases, including osteoporosis. To sum up, DLD is associated with OP through antioxidation and affects energy production in cell metabolism. In addition, DLD can also participate in the treatment of OP by promoting osseointegration.

### 3.9 The potential link between MTF1 and OP

MTF is a zinc finger protein that responds to and regulates various metals, especially zinc, at the cellular level (159–161). As a zinc sensor, MTF1 protects cells from oxidation and hypoxia by inducing the expression of antioxidants and other proteins (162, 163). Therefore, a significant relationship exists between MTF1 and redox metabolism (164). Zinc is needed in many biological

processes, such as enzyme activity, gene expression, cell cycle, apoptosis, and immunity (165–167). In addition, zinc can regulate the expression of SOCS3 by activating MTF1 to prevent inflammation (168). MTF1 is the only known transcription factor that mediates zinc-induced gene transcription (169). The content of available zinc in cells is directly related to the existence of metallothioneins (160). Metallothionein is involved in maintaining the balance of metal elements, such as copper, zinc, and cadmium (164); it binds zinc ions and regulates the balance of intracellular zinc ions, affecting many cellular processes, including gene transcription, apoptosis, proliferation, and differentiation (160); it also acts as a redox buffer (164). Metallothionein and metal balance regulation by MTF1 is related to heavy metal exposure (170). Zinc supplementation can activate the binding of MTF1 to DNA and initiate metallothionein mRNA transcription (171). The protein participates in the regular operation of mitochondria by regulating the permeability of the mitochondrial inner membrane (172). In addition, cadmium can be transformed into a non-toxic form by directly binding metallothioneins; metallothioneins can also inhibit cadmium toxicity by reducing cells' cadmium uptake and counteracting cadmium-induced ROS (173, 174). The accumulation of heavy metals makes MTF1 transfer from the cytosol to the nucleus (175, 176), and MTF1 can bind to the target gene promoter and promote the expression of metallothionein chelators, metal transporters and other metal-induced proteins (177, 178). The content of copper in the cells affects the binding of MTF1 to the target promoter (163). In addition, MTF1 nuclear translocation leads to a decrease in the level of consumable iron to prevent ferroptosis (179). Studies have shown that MTF-1 is the direct target of miR-148-3p, which regulates the transcriptional activity of MTF-1 (180).

In short, metal ions are necessary for the growth and metabolism of bone-related cells. MTF1 has a direct or indirect relationship with osteoporosis by controlling the balance of metal ions and redox, regulating the expression of related genes, and protecting against metal toxicity.

### 3.10 The potential link between LIPT1 and OP

LIPT1 encodes fatty acyltransferase 1 and participates in the lipoid acid metabolic pathway that regulates mitochondrial energy metabolism (181, 182). Among them, it can regulate the transport of LA, an essential substance in mitochondria (181). LA is involved in the TCA cycle, cellular energy metabolism, and glycine decomposition (183). Fatty acyltransferase 1 encoded by LIPT1 can transfer LA to the subunit of the 2-ketoacid dehydrogenase complex (183). If the homolog of LIPT1 disappears, it may lead to the decrease of lipoid of the E2 subunit of the 2-ketoacid dehydrogenase complex (183), and the lack of LIPT1 will inhibit the metabolism of the TCA cycle (184, 185). In addition, LIPT1 supports fat production and balances oxidizing and reducing glutamine metabolism (186).

In short, LIPT1 may be involved in osteoporosis by participating in the tricarboxylic acid cycle and affecting mitochondrial energy metabolism.

## 4 Conclusions and future perspectives

The essential micronutrient copper catalyzes various biological processes. Therefore, almost all cell types require copper for various physiological processes. Tsvetkov et al. recently proposed a new concept of “cuproptosis” and emphasized that it is a regulated form of cell death. Targeted copper toxicity has always been an effective method for treating tumor diseases, and the discovery of cuproptosis will undoubtedly attract more attention. The link between cuproptosis and osteoporosis may include the following. First, cuproptosis may be inhibited in several cells, and this inhibition promotes cell proliferation. Secondly, some critical genes regulating cuproptosis are associated with the developmental process of OP: PDHA1 regulates glycolysis and inflammation; PDHB can inhibit ERK signaling and regulate mitochondrial homeostasis, possibly affecting osteoclast differentiation; GLS mainly affects glutamine metabolism and antioxidant function; LIAS is essential in mitochondrial energy metabolism, mainly related to oxidative stress and inflammation; DLAT may affect OP mainly by influencing the conversion of pyruvate to acetyl-CoA in PDC and mitochondrial metabolism; FDX1 may be involved in osteoporosis through metabolism, immune cells, immune-related genes, and inflammation; CDKN2A is associated with cellular senescence; DLD is associated with osteoporosis through antioxidation, affecting energy production in cell metabolism and promoting bone integration; MTF1 has a direct or indirect relationship with osteoporosis by controlling the balance of metal ions and redox, regulating the expression of related genes, and protecting against metal toxicity; LIPT1 may participate in osteoporosis by participating in the tricarboxylic acid cycle and affecting mitochondrial energy metabolism.

Cuproptosis a recently discovered programmed cell death. Although studies on Cuproptosis have been published one after another, our team has collected and summarized the possible link between cuproptosis and osteoporosis for the first time. However, because the exact mechanism of cuproptosis is unclear, there are no direct reports of cuproptosis and osteoporosis in the clinic. However, there are direct reports of cuproptosis and osteosarcoma (187–189); cuproptosis-related genes and orthopedic-related diseases have been reported, such as rheumatoid arthritis and osteosarcoma (33, 190, 191). Copper levels are up-regulated in various cell types, including bone-related senescent cells and fibroblasts (192). It is one of the characteristics of cellular senescence (192–194); copper sulfate-induced premature senescence (CuSO<sub>4</sub>-SIPS) always mimics the molecular mechanism of replicative senescence, especially at the endoplasmic reticulum protein homeostasis level (195). Therefore, there is a need for more research to explore the mechanisms underlying cuproptosis that will likely provide new opportunities

for clinical application. Regarding therapeutic methods, the development of nanotechnology is exploiting nano-formulations with the cuproptosis mechanism to achieve functional regulation of cells by regulating the intracellular copper content through its delivery capacity. Combining cuproptosis and materials science may inspire a new class of highly effective anti-osteoporosis strategies.

Our team has been committed to studying exosomes, nanovesicles, and nanoparticles' targeted treatment of osteoporosis. Next, we plan to design a cuproptosis-targeting therapy for osteoporosis. Specific activators or inhibitors of cuproptosis depend on the selected cuproptosis-related genes and cell types. In short, we aim to provide a scientific basis for the clinical development of new strategies for treating osteoporosis; of course, this must be a hopeful and challenging exploration process.

## Author contributions

DL, HL, and XL generated the ideas and wrote the manuscript. ZG and QL participated in the discussions. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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