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REVIEW

Curculigoside is a Promising Osteoprotective Agent for Osteoporosis: Review

Jianqiang Du[1,2,*], Wenxiu Qin[1,*], Fayan Wen[3,*], Yandong Liu[3], Qi Zhang[4], Wangxin Liu[1,2], Chenyang Huang[1,2], Zhixiao Feng[1,2], Zhicheng Pan[4], Enpeng Gu[2]

[1]Departments of Graduate, Tianjin University of Traditional Chinese Medicine, Tianjin, People's Republic of China; [2]Department of Orthopedics, The Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, People's Republic of China; [3]Departments of Graduate, Gansu University of Traditional Chinese Medicine, Lanzhou, People's Republic of China; [4]Department of Orthopedics, Binhai New Area Hospital of Traditional Chinese Medicine and The Fourth Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, People's Republic of China

*These authors contributed equally to this work

Correspondence: Enpeng Gu; Zhicheng Pan, Email GU_19671213@163.com; 2286819448@qq.com

Abstract: The prevention and treatment of osteoporosis (OP) is one of the major issues in coping with the aging population; however, there are limited treatments available for OP. In-depth study of OP pathogenesis and development of new therapeutic strategies has become an urgent medical need facing the aging society. Curculigoside is a natural product widely found in plants, which can modulate cellular differentiation and function in osteogenic cells and exert significant osteoprotective effects. In addition, curculigoside showed significant positive effects on the treatment of OP animal models. Specific mechanisms include inhibition of inflammatory responses, antagonism of oxidative stress, and modulation of various signaling. Therefore, we hypothesized that curculigoside could represent a novel therapeutic strategy for OP. This article reviews recent research advances in the treatment of OP with curculigoside, including the origin and basic characteristics of curculigoside, the mechanisms and therapeutic effects that may be involved in in vitro as well as in vivo studies. We also examine the pharmacokinetics of curculigoside and investigate modified uses that may augment its therapeutic efficacy. This article seeks to encourage additional investigation into curculigoside-based treatments for osteoporosis.

Keywords: curculigoside, osteoporosis, mesenchymal stem cells, osteoblasts, osteoclasts, animal models

Introduction

Osteoporosis (OP) is a chronic metabolic bone disease characterized by decreased bone mineral density and impaired bone tissue microarchitecture.[1] The pathogenesis of OP is mainly due to abnormal changes in bone homeostasis between bone formation and bone resorption, eg, oxidative stress of bone-associated cells, chronic inflammation, and other factors can disrupt this dynamic equilibrium and lead to OP.[2,3] With the increasing aging of the population, OP has become an important public health problem facing the world. It has been reported that more than 200 million people suffer from OP worldwide.[4] About half of women and 20% of men over the age of 50 are at risk for osteoporotic fracture.[5] Currently, conventional pharmacologic regimens for OP include calcium supplements, hormone replacement therapy, and bisphosphonates, which can, to some extent, mitigate the progression of OP. However, long-term use presents risks of damaging the gastrointestinal tract, increasing blood clots, and seriously affecting patient adherence.[6] Therefore, there is an urgent need for a safe and effective treatment.

Herbal medicine has a long history of preventing and treating OP and has achieved certain results. Chinese herbal medicines have multi-target and multi-pathway regulatory effects, showing unique advantages in OP treatment. In addition, Compared with chemically synthesized drugs, herbal medicines are characterized by fewer adverse reactions, lower cost and more obvious effects in long-term use, which have broad application prospects. Curculigoside is a naturally occurring polyphenol compound that has a wide range of pharmacological effects and is used in the treatment of various diseases such as ulcerative colitis (UC),[7] Ischemic brain injury (IBI),[8] Alzheimer's disease (AD)[9] and

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rheumatoid arthritis (RA),[10] etc. OP as a common age-related skeletal disorder, is on the rise with the aging of society. However, effective therapeutic countermeasures are still lacking. As the main active ingredient of the traditional Chinese herb centella asiatica, curculigoside may be a potential option for the treatment of OP. Over the past decade. Curculigoside has shown significant osteoprotective effects in the OP field. Its molecular or cellular mechanisms include regulating the activity and differentiation of mesenchymal stem cells, osteoblasts and osteoclasts, reducing oxidative stress and inflammatory responses, etc, which helps to promote bone health, promises to be a safe and effective therapeutic drug. However, curculigoside still needs to be further developed in studies on the prevention and treatment of OP. Based on this, this article reviews the in vivo and in vitro experimental evidence that curculigoside is effective in ameliorating OP, aiming to emphasize its potential role in the prevention and treatment of osteoporosis, with a view to informing further studies on the prevention and treatment of OP.

Origin and Basic Characteristics of Curculigoside

Curculigo orchioides Gaertn is a perennial herb, one of the important Chinese herbs in the Chinese medicine system, widely distributed in Zhejiang, Guangxi, Sichuan, Yunnan, Guizhou, Taiwan and other provinces and regions of China, also produced in Japan and Southeast Asia and other regions.[11] For thousands of years, Curculigo orchioides Gaertn has been regarded as a commonly used traditional Chinese medicine in China and India, with the effect of nourishing the essence, tonifying the liver and kidneys, and has been used as an aphrodisiac and tonic, as well as for the treatment of impotence, jaundice, asthma, and bone-related diseases.[12] Modern research has shown that Curculigo orchioides Gaertn is rich in chemical constituents such as phenols, phenolic glycosides, lignans, etc.[13,14] Among them, curculigoside was identified as the main anti-OP active substance.[15] Curculigoside is a benzoic acid ester derivative, molecular formula is $C_{22}H_{26}O_{11}$, relative molecular mass is 466.44. Curculigoside monomer standard is white crystal, soluble in warm water, methanol, ethanol, n-butanol, acetone, etc, insoluble in petroleum ether, hashish, aether, and so on.[16]

Protective Effect of Curculigoside on Bone: An in vitro Test

Normal bone homeostasis is the result of the interaction between bone marrow mesenchymal stem cells (MSCs), osteoblasts and osteoclasts. As an important "executive" of bone metabolism in the human body, they are the key target cells in bone repair and bone reconstruction. We found that curculigoside, as an exogenous factor, has regulatory effects on mesenchymal stem cells, osteoblasts and osteoclasts of different origins. This includes regulating the balance between osteogenesis and lipogenesis, promoting angiogenesis, antagonizing oxidative stress and inhibiting inflammatory responses. Here, we summarize the data on the availability of curculigoside for the treatment of OP in in vitro studies (Table 1).

Regulatory Effects of Curculigoside on Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are pluripotent stem cells with self-renewal, multidirectional differentiation potential and immunomodulatory functions.[27] MSCs are present in almost all organs and tissues, such as bone marrow, adipose tissue, liver and cord blood.[28] MSCs have been shown to have good therapeutic potential in a variety of diseases such as diabetes mellitus complicated by severe limb ischemia,[29] skin defects,[30] fibrotic diseases,[31] and tumors.[32] In bone metabolic diseases, osteoblasts differentiated from MSCs play an important role in bone formation as well as remodeling processes.[33]

Curculigoside Regulates Bone Marrow Tissue-Derived Mesenchymal Stem Cells

Currently, BMSCs are the most commonly used MSCs, and BMSCs have potential for osteogenic and lipogenic differentiation. The balance between osteogenic and lipogenic differentiation is one of the important factors in regulating as well as maintaining bone homeostasis, and an imbalance of osteogenic/lipogenic differentiation may occur if the number, proliferative capacity, and differentiation potential of BMSCs decline due to aging or specific physiological conditions. Curculigoside, as a potential anti-OP natural active product, is able to exert osteoprotective effects by modulating the differentiation pathway of BMSCs. Protective effects.

Table 1 Effects of Curculigoside in Regulating Different Cells

Cell Model	Dose	Optimal Dose	Results	Mechanisms/ Pathways	References
BMSCs	10, 100, 500 μm	100 μm	Promotes proliferation, osteogenic differentiation and mineralization	Adjustment of the OPG system	[17]
BMSCs	10, 100, 1000 μm	100 μm	Promotes osteogenesis and inhibits lipogenesis	Regulating the MEK-ERK signaling pathway	[18]
ADSCs	1, 2.5, 5, 10, 20 μmol/L	5 μmol/L	Promotes proliferation, osteogenic differentiation and mineralization	Regulation of the PI3K/AKT signaling pathway	[19]
AFSCs	1, 10, 100, 200 mg/mL	100 mg/mL	Promotes proliferation and osteogenesis while inhibiting osteoclast formation	Regulation of the Wnt/β-catenin signaling pathway	[20]
MC3T3-E1 cells	10, 20, 50, 100 μg/mL	100 μg/mL	Promotes angiogenesis and osteogenic differentiation	Regulation of the VEGF/Flt-1 signaling pathway	[21]
H_2O_2-induced osteoblasts	0.1, 1, 10 μM	10 μM	Reducing oxidative stress and promoting osteogenic differentiation	Regulation of the p38MAPK, ERK, NF-kB signaling pathways	[22]
H_2O_2-induced MC3T3-E1 cells	10^{-6}, 10^{-7}, 10^{-8} mol·L^{-1}	10^{-6} mol·L^{-1}	Reducing oxidative stress and promoting osteogenic differentiation	Regulation of the FoxO1 signaling pathway	[23]
Excess iron-induce MC3T3-E1 cells	10 μM	10 μM	Reduces oxidative stress and promotes autophagy	Regulation of the IGFR/AKT signaling pathway	[24]
Dexamethasone-induced osteoblasts	25, 50, 100 μg/mL	100 μg/mL	Reduces inflammatory factors and promotes proliferation and differentiation of osteoblasts	Suppression of the inflammatory response	[25]
RANKL and H_2O_2-induced RAW264.7 cells	1, 5, 10 μmol·L^{-1}	10 μmol·L^{-1}	Reduces oxidative stress and inhibits osteoclastogenesis	Regulation of the I Nrf2/NF-κB signaling pathway	[26]

Shen et al[17] set three groups of different concentrations of curculigoside to intervene on BMSCs, and found that cells cultured in curculigoside grew faster than those cultured in the control group, which had a pro-proliferative effect on BMSCs. After 7 days of culture, the alkaline phosphatase (ALP) activity in BMSCs cultured in the three concentrations of curculigoside was approximately twice as high as that in the control group. Meanwhile, curculigoside treatment significantly enhanced the expression of collagen type 1 (Col1), osteocalcin (OCN), osteoprotegerin (OPG), and Runt-related transcription factor 2 (RUNX2) in BMSCs, especially at a dose of 100 μm. In addition, this study showed that curculigoside could directly stimulate osteoprotegerin (OPG) production by BMSCs, suggesting that curculigoside may inhibit osteoclastogenesis by regulating the OPG system, but the study has not yet elucidated the underlying mechanism. Transcriptional co-activator (TAZ) with a PDZ-binding motif has been shown to be a nuclear transcription factor that plays a key role in stem cell differentiation.[34] TAZ binding to RUNX2 promotes osteogenesis and interacts with peroxisome proliferator-activated receptorγ (PPARγ) to inhibit adipogenesis.[35] Yu et al[36] reported that PGC-1α-targeted TAZ could alter the bone-fat balance during skeletal aging. Previously, Wang et al[37] indicated that the MEK-ERK pathway was associated with TAZ during osteogenic differentiation of BMSCs. It was subsequently found that appropriate concentrations of curculigoside could up-regulate the p-ERK/ERK ratio, significantly increase the expression of TAZ, RUNX2, and OCN in BMSCs, and down-regulate the protein and mRNA levels of PPARγ, a marker of adipogenesis, during osteogenesis, thus stimulating the osteogenic ability and inhibiting the adipogenic ability of BMSCs, and reversing the osteogenic/adipogenic Imbalance of osteogenic/lipogenic differentiation.[18] In contrast, TAZ knockdown or use of MEK-ERK pathway inhibitors reduced this regulatory effect of curculigoside. Notably, the optimal concentration of curculigoside to induce differentiation of BMSCs up to was 100 μm, which is consistent with the results of Shen et al.[17]

Curculigoside Regulates Adipose Tissue-Derived Mesenchymal Stem Cells

Adipose-derived stem cells (ADSCs) are a class of pluripotent stem cells with multidirectional differentiation potential isolated from adipose tissue, which are mesenchymal stem cells. In recent years, it has been found that compared with BMSCs, ADSCs are easier to isolate, more abundant and more productive.[38] Since the proliferation and differentiation of

ADSCs are less affected by age and multiple passages, making them a potential source of cell-based therapies.[39] Similarly, ADSCs have a high potential to differentiate into multilineage cells, including osteoblasts, chondrocytes, and adipocytes.[40–42]

You and Xu[19] treated ADSCs with different concentrations of curculigoside, and found that curculigoside enhanced ALP activity and calcium deposition, and significantly up-regulated the expression of RUNX2, ALP and Osterix. In addition, the PI3K/AKT signaling pathway has been shown to be critical in all stages of osteoblast differentiation and maturation, bone development and growth.[43] In this study, curculigoside demonstrated the ability to significantly upregulate the phosphorylation of AKT to P-AKT to promote osteogenic differentiation in ADSCs, these promotional effects were reversed by the PI3K/AKT signaling pathway inhibitor LY294002. However, which receptors or proteins are associated with the role of curculigoside in ADSCs is not clear, and thus more studies are needed. Furthermore, there is an inverse relationship between osteogenic and lipogenic differentiation in ADSCs,[44] inhibition of adipogenesis and specificity in promoting osteogenesis provide a possible novel therapeutic approach for the treatment of osteoporosis.[45]

Curculigoside Regulates Human Amniotic Fluid-Derived Mesenchymal Stem Cells

Amniotic fluid-derived stem cells (AFSC) have a strong potential to differentiate into osteoblasts and to become a novel cell source for the treatment of bone diseases.[46,47] AFSC have a MSC phenotype with the ability to migrate and engraft into a wide range of musculoskeletal tissues (especially injury sites) and undergo site-specific osteogenic differentiation.[48] Amniocentesis is a widely accepted procedure for prenatal testing with low risk to both mother and fetus, and AFSC can be easily obtained.[49] In addition, AFSC has no ethical implications for clinical use and has no tumorigenic risk.[50] Thus, AFSC has emerged as an ideal candidate for cell-based therapies with the promise of improving bone formation in patients suffering from a variety of metabolic and genetic bone diseases, including osteoporosis.

The results of Liu et al[20] showed that curculigoside dose-dependently promoted ALP activity and calcium deposition during osteogenic differentiation of human amniotic fluid-derived mesenchymal stem cells (hAFSCs) within a certain concentration range. As analyzed by RT-PCR, curculigoside upregulated Col1 and osteoblast protein (OPN). Meanwhile, the ratio of OPG to RANKL was increased, suggesting that curculigoside inhibited osteoclastic differentiation of hAFSCs. The classical Wnt signaling pathway is involved in the osteogenic differentiation of MSCs.[51] Wnt signaling promotes osteoblast differentiation through β-catenin (β-catenin) activation,[52] and knockdown of the β-catenin gene at early stages of development leads to aberrant osteoblast differentiation.[53] In a study by Liu et al,[20] curculigoside upregulated the mRNA expression of β-catenin and cell cycle proteins and promoted osteogenic differentiation of hAFSCs. And DKK-1 (a specific inhibitor of β-catenin signaling) significantly inhibited the above effects. Thus, it is clear that curculigoside can promote hAFSC osteogenic differentiation and has potential application in the treatment of bone diseases.

Regulation of Osteoblasts by Curculigoside

During normal bone metabolism, osteoblasts mediate bone formation and osteoclasts mediate bone resorption, and both maintain a dynamic balance. Osteoblasts are the cell type that plays a direct role in bone formation in the osteogenic spectrum of cells, and are able to secrete bone matrix proteins including COLI, OPN and OCN, etc.[54] In addition, RANKL secreted by osteoblasts promotes osteoclast recruitment, differentiation, activation, and survival by binding to RANK, a specific receptor on the surface of osteoclasts. Osteoblasts also secrete OPG, a soluble receptor for RANKL, which inhibits osteoclast differentiation and activity by preventing RANK/RANKL binding.[55] Based on the above properties, osteoblasts are essential for the balance of bone metabolism, bone development, fracture repair, and bone remodeling. Curculigoside is a potential osteoblast promoter that promotes the proliferation and differentiation of osteoblasts, as well as the formation of bone nodules.[56]

Ma et al[21] found that curculigoside significantly enhanced the proliferation of mouse osteoblast precursor MC3T3-E1 cells and stimulated the production of vascular endothelial growth factor (VEGF), Fms-like tyrosine kinase-1 (Flt-1) and bone morphogenetic protein-2 (BMP-2). VEGF, as an important angiogenic factor, not only mediates bone angiogenesis but also influences the differentiation of progenitor cells into osteoblasts to stimulate bone repair.[57] Flt-1, also known as VEGF receptor 1, is another factor that plays an important role in vascular

maintenance, endothelial precursor recruitment.[58] Mice deficient in Flt-1 exhibit lower bone conversion rates at an early age, suggesting that Flt-1 signaling is important not only for osteogenic cell differentiation but also for osteoblast activity during early stages of growth.[59] In conclusion, the results of these studies and the data from the current study suggest that the role of curculigoside for bone protection is related to the VEGF/Flt-1 system. Oxidative stress due to high ROS levels is considered to be a major cause of various degenerative diseases including osteoporosis.[60] H_2O_2 is one of the major reactive oxygen species, and H_2O_2 stimulates ERK-dependent NF-κB activation, leading to impaired osteoblast differentiation.[61] Curculigoside can increase superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities by regulating the phosphorylation of p38MAPK, ERK, and NF-kB pathways, and can significantly restore ALP activity, calcium deposition, and RUNX2 levels in osteoblasts under H_2O_2-induced oxidative stress, as well as increase the expression of COLI.[22] It was shown that curculigoside promotes the differentiation of osteoblasts under oxidative stress and contributes to the amelioration and prevention of OP. In addition, the body can regulate the transcription level of forkhead transcription factor 1 (FoxO1) under hyperoxia to increase the activity of SOD and catalase (CAT) to counteract the damage of oxidative stress on bone.[62] Curculigoside enhances the capacity of osteoblasts to resist oxidative damage by regulating FoxO1a expression and nuclear translocation to increase proliferation, differentiation and bone mineralization nodule formation in H_2O_2-injured osteoblasts.[23] Zhang et al[24] found that curculigoside up-regulated the levels of FoxO1 and nuclear factor E2-related factor 2 (Nrf2), down-regulated the levels of p53 and the levels of FoxO1 phosphorylation, and promoted FoxO1 nuclear translocation by inhibiting the IGFR/AKT signaling pathway, which in turn improved cellular autophagy and reduced the MC3T3-E1 cell apoptosis triggered by hyperoxia. Zhu et al[25] found that curculigoside not only increased the mitochondrial membrane potential (MMP) level and decreased ROS generation in osteoblasts under dexamethasone (DEX)-induced osteoclasts, but also inhibited the release of inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and COX-2, to protect osteoblasts injured by DEX. These results provide new insights for further investigation of the osteoblast protective mechanism of curculigoside.

Regulation of Osteoclasts by Curculigoside

Osteoclasts are specialized multinucleated cells differentiated from hematopoietic stem cells that are primarily responsible for bone resorption and maintain bone metabolic homeostasis in conjunction with osteoblast-mediated bone formation.[63] However, excessive bone resorption usually leads to an imbalance in bone homeostasis, resulting in net bone loss and ultimately OP.As mentioned previously, oxidative stress affects the process of osteoblast differentiation.[61] Similarly, there is growing evidence that oxidative stress is involved in osteoclastogenesis.[64] Excessive ROS production activates NF-κB and MAPK pathways and induces osteoclastogenesis.[64] Activated NF-κB activates T-cell cytoplasmic nuclear factor 1 (NFATc1), which is responsible for the regulation of genes encoding tartrate-resistant acid phosphatase (TRAP) and histone K (Ctsk), and subsequently increases osteoclast formation and bone resorption.[65] Nrf2 is a major transcription factor that controls the gene expression of many cytoprotective enzymes, such as heme oxygenase-1 (HO-1), quinone reductase (NQO-1), and γ-glutamylcysteine synthetase (GCS), with antagonistic effects on oxidative stress.[66] Therefore, drugs with antioxidant activity and modulation of Nrf2, NF-κB signaling pathways may be able to inhibit osteoclast formation and differentiation to prevent and treat OP.

In a study by Liu et al,[26] they found that curculigoside inhibited TRAP activity induced by RANKL and H_2O_2 in osteoclasts, and decreased the release of bone fragment degradation products and the expression of matrix metallopeptidase 9 (MMP9). In addition, curculigoside inhibited ROS levels in osteoclasts. What's more, curculigoside enhanced Nrf2 expression and nuclear translocation and decreased NF-κB expression and p65 phosphorylation and nuclear translocation in osteoblasts. And Nrf2 inhibitor ML385 and NF-κB inhibitor Bay11-7082 antagonized the above effects of curculigoside. Therefore, it is suggested that attenuating oxidative stress and osteoclastogenesis by activating Nrf2 and inhibiting the NF-κB pathway is a potential mechanism for curculigoside treatment of OP.

Protective Effect of Curculigoside on Bone: In vivo Experiments

The construction and selection of appropriate animal models is not only an important foundation for the in-depth study of the pathogenesis of OP, but also an effective means for clinical screening and comprehensive evaluation of drugs against

OP. Researchers have established various animal models to evaluate the therapeutic value of curculigoside according to the pathogenesis of OP. These models include the animal model of OP caused by ovariectomy, [19,67] Glucocorticoids cause OP in animal models [68,69] and the OP animal model of natural aging.[18] The osteoprotective effect of curculigoside in animal models of gene-induced Alzheimer's disease (AD) has also been explored,[70] In addition, Zhu et al [71] explored the interventional effects of curculigoside in a mouse model of cranial capitellar osteolysis, exemplifying the promising application of curculigoside in bone tissue engineering. Here, we summarize the data collected from curculigoside studies on the availability of several of these animal models (Table 2).

Bone Protection by Curculigoside in a Postmenopausal OP Model

Ovariectomized animal model is the most commonly used classical model to simulate postmenopausal osteoporosis. You et al[19] established an OVX mouse osteoporosis model by removing bilateral ovaries. The results showed that the bone trabeculae in the OVX model group were sparse and reduced compared with those in the sham-operated group, the expression of RUNX2 was significantly down-regulated, and the expression of p-AKT was lower in osteoblasts. However, treatment with curculigoside significantly reversed these conditions. Similarly, Wang et al[67] used bilateral ovariectomy to establish a rat model of osteoporosis, and treatment with curculigoside was able to significantly increase the elevated serum OCN and ALP levels, and decrease the levels of TNF-α and IL-6 in rats.Micro-CT showed that curculigoside contributed to increased bone mineral density, trabecular bone thickness, number of trabeculae, and bone volume fraction were elevated. It has been reported, Long-stranded noncoding RNA (lncRNA) KCNQ1 overlapping transcript 1 (KCNQ1OT1) expression levels were significantly reduced in tissue samples from OP patients,[72] Upregulation of miR-214-5p expression in bone marrow mononuclear cells from OP patients may promote osteoclast differentiation and exacerbate bone loss,[73] And bioinformatics showed that lncRNA KCNQ1OT1 had some targeting relationship with miR-214-5p. When curculigoside acted on OP rats, lncRNA KCNQ1OT1 expression was up-regulated and miR-214-5p expression was down-regulated in rat femur tissues, however, the KCNQ1OT1 inhibitor reversed this effect. These results suggest that the lncRNA KCNQ1OT1/miR-214-5p axis may be a potential mechanism by which curculigoside promotes osteogenesis and improves OP symptoms.

Table 2 Interventional Effects of Curculigoside on Different Animal Models

Animal Model	Intervention	Drug Dose	Time	Results	Mechanisms/Pathways	References
Ovariectomize-induced C57BL/6 mice	Intraperitoneal injection	7.5 mg/kg	4 weeks	Bone microarchitecture improvement	Regulation of the PI3K/AKT signaling pathway	[19]
Ovariectomize-induced SD rats	Intraperitoneal injection	5 mg/kg	14 weeks	Bone microarchitecture improvement	Regulation of the lncRNAKCNQ1OT1/miR-214-5p signaling pathway	[67]
Dexamethasone-induced SD rats	Oral	15 mg/kg	8 weeks	Increased autophagic activity, bone microstructure restoration	Regulation of the lncRNA MEG3/miR-181a-5p signaling pathway	[68]
Dexamethasone-induced C57BL/6 mice	Oral	5, 45 mg/kg	8 weeks	Increased bone density and decreased number of osteoclasts	Increased expression of osteogenic differentiation-related proteins in osteoblasts	[69]
18-month-old C57BL/6 mice	Oral	50, 100 mg/kg/d	2 months	Reduction of fat cells and improvement of bone microarchitecture	Regulating the MEK-ERK pathway	[18]
APP/PS1-induced C57BL/6J mice	Oral	100 mg/kg	4 weeks	Improvement of bone microstructure and its mechanical properties	Antioxidant	[70]
Ti-induced C57/BL6 mice	Intraperitoneal injection	20, 40 mg/kg/d	14 days	Parameters related to the reduction of osteolysis	Promotes osteoblast differentiation and reduces osteoclast formation	[71]

Bone Protection by Curculigoside in the Glucocorticoid OP Model

Glucocorticoids are widely used to treat a variety of inflammatory and autoimmune diseases, with glucocorticoidogenic OP being one of their toxic side effects. Wang et al[68] used intramuscular dexamethasone sodium phosphate to induce the establishment of an OP model in SD male rats, and the results of treatment with curculigoside showed that the morphological evaluation of tibial bone in rats improved significantly compared with that of the model group, and it was further found that the expression of LC3II/I, Beclin1, miR-181a-5p increased, and the lncRNA MEG3 expression was decreased, suggesting that curculigoside may promote autophagy activity of rat osteoblasts by regulating the LncRNA MEG3/miR-181a-5p signaling axis, thus exerting an osteoprotective effect.[74,75] Similarly, Han et al[69] found that curculigoside intervention significantly reduced SOD content in serum, increased SOD and CAT content, and attenuated oxidative stress levels, which had a modulating and ameliorating effect on osteoporosis symptoms in mice compared to dexamethasone-induced mice in the model group. It was further found that curculigoside could promote the expression of osteogenic differentiation-related proteins. In conclusion, the above results preliminarily demonstrated that curculigoside had good effects in the glucocorticoid-induced osteoporosis treatment group.

Bone Protection by Curculigoside in a Geriatric Model of OP

The aging mouse model can well simulate the process of age-related OP bone loss. Compared with 3-month-old mice, 18-month-old mice showed a significant decrease in femoral trabecular volume fraction, trabecular thickness and trabecular number, and a significant increase in trabecular spacing. Also, significant accumulation of fat in bone marrow with age was confirmed by more osmium tetroxide (OsO4)-stained lipid droplets in bone marrow.[18] It was found that oral administration of curculigoside to 18-month-old mice significantly increased bone mass and decreased the number of adipocytes in the bone marrow in senescent mice. It was further found that curculigoside regulated TAZ expression through the MEK-ERK pathway to promote osteogenesis at the expense of reducing adipogenesis in BMSC and ameliorate bone loss in senescent mice. This provides a rationale for the clinical application of curculigoside in the treatment of senile osteoporosis.[18]

Bone Protection by Curculigoside in a Model of Alzheimer's Disease Combined with OP

Alzheimer's disease (AD) and OP are both common chronic degenerative diseases in the elderly population, and epidemiology shows a very high co-morbidity between the two diseases.[76] In addition, there may be common pathogenic factors between AD and OP, among which amyloid-β (Aβ) is one of the most widely investigated ones.Aβ is neurotoxic, leading to impaired synaptic plasticity and neuronal apoptosis, accelerating the rate of cognitive decline; at the same time, Aβ deposition in the brain also exacerbates the deposition of Aβ through a series of signaling, thus creating a vicious circle.[77] Previous studies have observed that Aβ42, one of the isoforms of Aβ, is expressed at higher levels than controls in both osteoporotic human and animal bone tissues, and is negatively correlated with bone mineral density levels, suggesting that Aβ42 may enhance osteoclast differentiation and activation.[78] APP/PS1 mutant transgenic mice are one of the common AD models. Compared with wild-type mice, APP/PS1 mice showed significantly increased levels of Aβ40 and Aβ42 in the brain and femur, and in addition, the elevated levels of Aβ led to increased levels of the bone resorption marker, histone K, and decreased levels of the biochemical marker of bone formation, osteocalcin. Serum levels of markers of osteoclast activity (TRACP5b), IL-6, and TNF-α were significantly higher in APP/PS1 mice than in wild-type mice. IL-6 and TNF-α levels were significantly higher than those of wild-type mice, and IL-6 and TNF-α not only directly stimulated osteoclastogenesis and bone resorption, but also synergistically stimulated RANKL production in osteoblasts.[79] Zhao et al[70] intervened APP/PS1 mice with curculigoside, which was able to reverse the above alterations, significantly improve the academic performance of APP/PS1 mutant transgenic mice, and improve bone loss. It suggests that curculigoside is highly likely to be a potential therapeutic drug for patients with AD combined with OP.

Osteoprotection by Curculigoside in a Model of Cranial Capitellar Osteolysis

Total Joint Arthroplasty (TJA) offers significant benefits in terms of joint pain relief and joint function restoration in patients with bone and joint disorders.[80] However, TJA failure can occur due to infection, prosthesis fracture or loosening and wear. Of these, aseptic prosthetic loosening accounts for 38% of cases, with the majority of aseptic loosening being caused by particle abrasion,[81,82] This induces osteolysis, which has become a major problem limiting the longevity of artificial joints.

In view of the advantages that curculigoside embodies in bone protection, Zhu et al[71] chose titanium particles (Ti) to simulate periprosthetic osteolysis in vivo and then investigated the effect of curculigoside on osteolysis. They first prepared a model of Ti induced osteolysis in the mouse skull by surgical means, and gave mice intraperitoneal injection of curculigoside for intervention on the 2nd day, and then took cranial bone tissues for further experiments after executing each group of mice after 14 days. The results showed that titanium injection induced osteolysis and bone loss in the cranial bones of the mice, and the cranial bones of the mice exhibited osteolysis characteristics. In addition, significant changes in osteolysis-related parameters, including BMD, BV/TV, and Tb.Th, occurred in the Ti group compared with the sham-operated group. However, in the curculigoside-treated group, the extent of Ti-induced osteolysis was attenuated and also reversed osteolysis-related parameters. In addition, Ti injection increased the expression of IL-1β, IL-6, RANKL, and TNF-α and suppressed the expression of OPG in mouse cranium, which increased the production of pro-inflammatory cytokines and the RANKL/OPG ratio, leading to bone destruction. In contrast, curculigoside significantly reversed these changes and promoted bone formation. In summary, curculigoside can effectively alleviate attenuate titanium-induced osteolysis, which is expected to provide curculigoside as a potential bone protective agent for the prevention and treatment of bone defects.

Pathway Related to the Effect of Curculigoside on Bone

As the study of OP molecular pathology network continues to deepen, its intrinsic cellular signaling mechanisms have been gradually and systematically elucidated. It is worth noting that curculigoside has the characteristics of multi-pathway regulation, and it can intervene in key signaling axes such as OPG/RANKL/RANK, PI3K/Akt, Wnt/β-catenin, MAPKs, Nrf2, and NF-κB, etc (Figure 1). Curculigoside has the advantage of multi-targeting in bone metabolism homeostasis. Advantage lays an important theoretical foundation for its precise therapeutic strategy and new drug development based on signaling pathways.

The OPG/RANKL/RANK signaling pathway has been found to regulate osteoclast differentiation, induction, activation and maintenance and is recognized as a potential target for the treatment of OP.[83] In this pathway, RANKL binds to

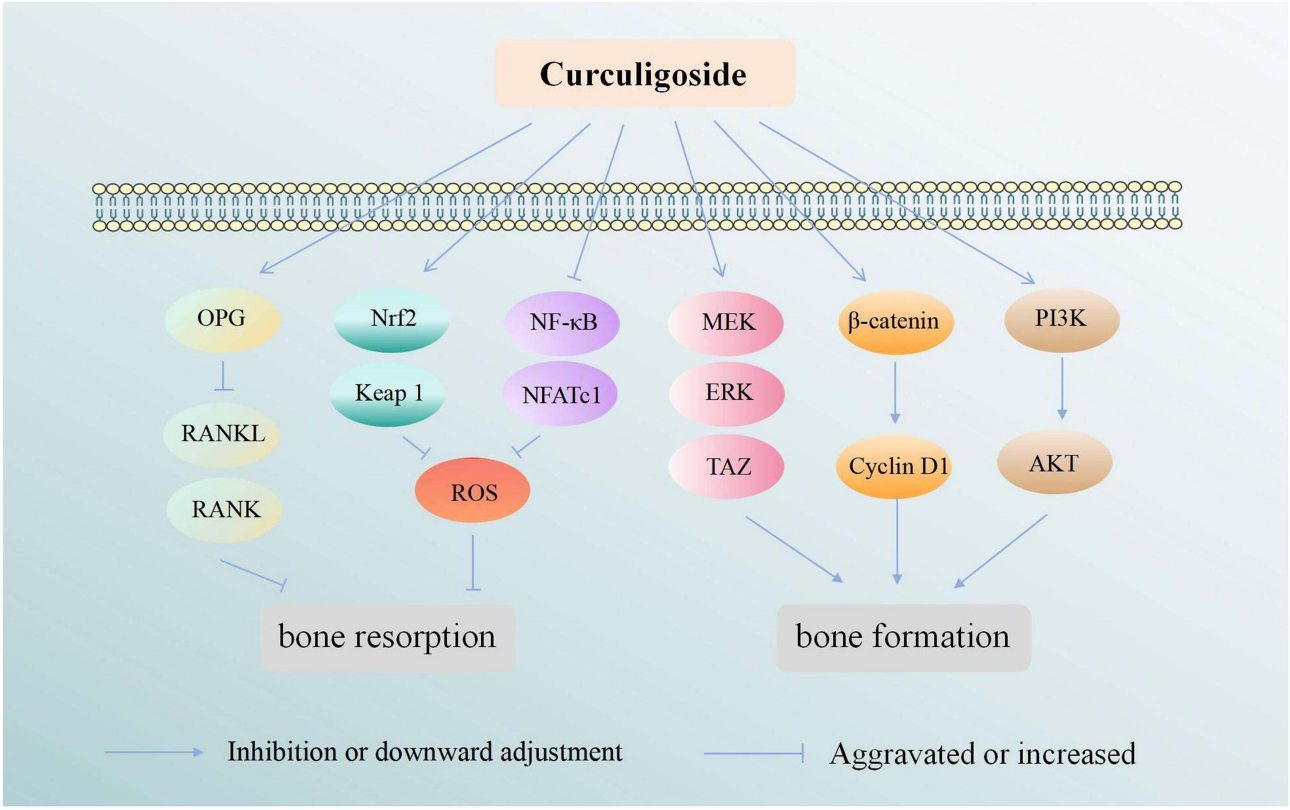

Figure 1 Pathway related to the effect of curculigoside on bone.

RANK on the surface of osteoclasts and will form a RANKL-RANK complex and stimulate osteoclast activation, formation, and differentiation.[84] OPG, a decoy receptor produced primarily by osteoblast-profiling cells, inhibits osteoclast formation.[85] Specifically, OPG regulates osteoclasts to inhibit bone resorption and delay bone loss by competitively binding to RANKL, thereby preventing RANKL-RANK complex formation. Normally, under normal conditions, OPG and RANKL are in relative equilibrium, and if the level of RANKL/OPG is elevated, it directly affects osteoclasts.[86] The experimental study[17] found that curculigoside synergistically promoted bone formation and inhibited bone resorption by up-regulating Runx2 and OPG expression and inhibiting osteoclast activity through regulating OPG/RANKL balance. Similarly, consistent results were obtained by Liu et al.[20]

Phosphoinositide 3-kinase (PI3K) is a lipid kinase found in the cytoplasm, and serine/threonine kinase (AKT) is an essential signaling target downstream of PI3K.[87] Activation of PI3K results in the phosphorylation of phosphatidylinositol 2-phosphate (PIP2) to PIP3, which causes a conformational change and translocation of Akt to the cell membrane and exposure of the activation site, which is then activated by PI3K, leading to complete phosphorylation of Akt.[88] Activated AKT affects downstream signaling molecules such as mammalian target of rapamycin palladium protein (mTOR) and participates in the regulation of cellular life activities. Several studies have demonstrated that the PI3K/AKT signaling pathway, once activated, significantly promotes the proliferation and differentiation of osteoblasts and plays an important role in regulating and maintaining the homeostasis of bone tissue.[89,90] You et al[19] combined in vivo as well as in vitro experiments to demonstrate that curculigoside significantly increased the phosphorylation levels of PI3K and AKT (p-PI3K and p-AKT) and promoted bone remodeling. In contrast, this osteoprotective effect of curculigoside was partially reversed using the PI3K inhibitor LY294002.

The Wnt/β-catenin pathway is recognized as a classical pathway in the Wnt signaling pathway. The key to the activation of this pathway lies in the binding of Wnt ligands to specific curculigoside and co-receptors, which triggers a series of intracellular events that stabilize β-catenin and promotes the transcription and expression of related target genes.[91] The Wnt/β-catenin pathway plays a key role in osteoblast differentiation, bone remodeling and skeletal homeostasis. Studies have shown that curculigoside activates the Wnt/β-catenin pathway to enhance the osteogenic differentiation of hAFSCs by up-regulating the expression of β-catenin and Cyclin D1, providing a molecular basis for its stem cell-based bone regeneration therapy.[20]

The mitogen-activated protein kinase (MAPK) signaling pathway consists of three core members, namely, extracellular signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38MAPK. MAPK signaling is one of the key pathways in the eukaryotic signaling network, which mediates cellular responses to a variety of stimuli and is closely related to inflammation, apoptosis, growth and differentiation.[92] The MEK-ERK pathway was first recognized in the early 1990s as a member of the MAPK family.[93] Activation of p-ERK was previously shown to be critical for RUNX2 upregulation and transcriptional activity.[94] Wang et al[18] found that curculigoside mediates osteogenic differentiation of BMSCs by upregulating TAZ signaling through the MEK-ERK pathway.

There is growing evidence that ROS accumulate in bone with age and estrogen deficiency, and that excess ROS can activate the NF-κB pathway and induce osteoclastogenesis.[64] Specifically, NF-κB activation induces nuclear factor activation in activated NFATc1, which is responsible for the regulation of genes encoding TRAP and Ctsk, and subsequently increases osteoclastogenesis and bone resorption.[65] Nrf2 is a major endogenous antioxidant defense regulator that controls the gene expression of many cytoprotective enzymes, such as HO-1 and gamma-glutamylcysteine synthetase (GCS), to counteract oxidative stress.[66] Therefore, drugs with antioxidant activity and the ability to modulate Nrf2 and NF-κB signaling pathways may be able to inhibit osteoclast formation and differentiation. The study[26] has shown that Curculigoside effectively inhibits osteoclastogenesis and bone resorption function and alleviates oxidative stress injury by activating the Nrf2/Keap1 pathway and inhibiting the NF-κB pathway, providing a novel candidate molecule for OP therapy.

Pharmacokinetics and Modified Applications of Curculigoside

Given the wide range of pharmacological activities of curculigoside and its applications, it is important to understand the pharmacokinetics and in vivo behavior of curculigoside, which is rapidly distributed to multiple tissues after oral administration, including the heart, lungs, spleen, intestines, stomach, kidneys, liver, brain, and bone marrow; however,

the amount absorbed into the systemic circulation is relatively small.[95] The absolute bioavailability of oral curculigoside was 0.38%, 0.22%, and 0.27% at 100, 200, and 400 mg/kg, respectively, and the distribution and clearance of curculigoside in vivo following intravenous administration was essentially the same as for oral administration.[95] This result is similar to a previous report where Zhao et al[96] reported that the absolute bioavailability of curculigoside was also only 1.27% after oral administration of 32 mg/kg dose of curculigoside to rats. Therefore, it can also be concluded that curculigoside may have a low absolute bioavailability. This will greatly hinder its pharmacological action and clinical application, therefore, improving the bioavailability of curculigoside is an important research topic in its development.

Studies have shown that co-administration with verapamil enhances the absorption of some natural compounds with poor oral bioavailability, as verapamil, by inhibiting the activity of P-gp and some CYP enzymes, increases the absorption of the drug in the intestine and slows down the rate of its elimination in the liver.[97] Wang et al[98] administered verapamil (10 mg/kg) pretreated and unpretreated rat curculigoside (20 mg/kg) orally, respectively; verapamil increased the peak plasma concentration of curculigoside from 60.17 ng/mL to 93.66 ng/mL and inhibited the exocytosis of curculigoside and increased the uptake of curculigoside in Caco-2 cells. Rat liver microsomal incubation experiments demonstrated that verapamil significantly reduced the intrinsic clearance of curculigoside (from 38.8 to 23.6 μL/min/mg protein) and effectively increased bioavailability. Recent studies have discussed the protective effects of delivering senecioside to modulate bone reconstruction through three-dimensional (3D) printing of bionic hydroxyapatite composites. Weng et al[99] developed a bionic composite scaffold (HGSC) loaded with senecioside by utilizing 3D printing technology. Eight weeks after implantation of HGSC in cranial defects in rats, micro-computed tomography and histological observations showed significant angiogenesis and new bone growth in the area treated with HGSC composite scaffold. It is noteworthy that the composite stent simultaneously achieved sustained drug release for up to 12 days, which significantly improved the drug utilization of senecioside. This is an exciting news, which provides a solid theoretical foundation for Xianmaooside in clinical bone repair.

Conclusion

Over the years, natural products have shown great potential in the prevention and treatment of osteoporosis, leading to the emergence of more acceptable complementary alternatives that are less costly, have fewer side effects, and are more widely used in the long term.[100–102] According to our findings, curculigoside acts on osteoblasts, osteoclasts, and mesenchymal stem cells through a variety of mechanisms, which can reverse bone loss and protect bone homeostasis. In addition, evidence from various animal models confirms the therapeutic effects of curculigoside on bone destruction, which will likely provide a basis for the therapeutic use of curculigoside to be transferred to clinical practice. However, this therapeutic approach is still in the early stages of clinical translation, and there are still many obstacles between its experimental results and clinical application. For example, the absorption and metabolic stability of curculigoside is not ideal, and the drug delivery route as well as bioavailability still need to be optimized, which is an urgent issue. Overall, as a readily available and inexpensive natural compound, curculigoside has a favorable osteoprotective effect. Therefore, we hypothesize that curculigoside is expected to be a potential alternative therapy for the treatment of OP, and the application of curculigoside in bone tissue engineering is also expected.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Compston JE, Mcclung MR, Leslie WD. Osteoporosis. *Lancet*. 2019;393(10169):364–376. doi:10.1016/S0140-6736(18)32112-3
2. Badila AE, Radulescu DM, Ilie A, Niculescu A, Grumezescu AM, Radulescu AR. Bone regeneration and oxidative stress: an updated overview. *Antioxidants-Basel*. 2022;11(2). doi:10.3390/antiox11020318
3. Clowes JA, Riggs BL, Khosla S. The role of the immune system in the pathophysiology of osteoporosis. *Immunol Rev*. 2005;208(1):207–227. doi:10.1111/j.0105-2896.2005.00334.x
4. Ballane G, Cauley JA, Luckey MM, El-Hajj Fuleihan G. Worldwide prevalence and incidence of osteoporotic vertebral fractures. *Osteoporos Int*. 2017;28(5):1531–1542. doi:10.1007/s00198-017-3909-3
5. Rizzoli R, Chevalley T. Nutrition and osteoporosis prevention. *Curr Osteoporos Rep*. 2024;22(6):515–522. doi:10.1007/s11914-024-00892-0
6. Cheng C, Wentworth K, Shoback DM. New frontiers in osteoporosis therapy. *Annu Rev Med*. 2020;71(1):277–288. doi:10.1146/annurev-med-052218-020620
7. Li F, Huang H, Zhao P, et al. Curculigoside mitigates dextran sulfate sodium-induced colitis by activation of KEAP1-NRF2 interaction to inhibit oxidative damage and autophagy of intestinal epithelium barrier. *Int J Mol Med*. 2023;52(5). doi:10.3892/ijmm.2023.5310
8. Xu Y, Wen K, Liu A, Wang X, Xu H, Wen H. Efficacy of curculigoside in protecting against ischemic brain injury through regulation of oxidative stress and NF-kappaB and PI3K/Akt expression. *J Ethnopharmacol*. 2023;301:115804. doi:10.1016/j.jep.2022.115804
9. Gong Y, Wang Y, Li Y, Weng F, Chen T, He L. Curculigoside, a traditional Chinese medicine monomer, ameliorates oxidative stress in Alzheimer's disease mouse model via suppressing ferroptosis. *Phytother Res*. 2024;38(5):2462–2481. doi:10.1002/ptr.8152
10. Tan S, Xu J, Lai A, et al. Curculigoside exerts significant anti-arthritic effects in vivo and in vitro via regulation of the JAK/STAT/NF-kappaB signaling pathway. *Mol Med Rep*. 2019;19(3):2057–2064. doi:10.3892/mmr.2019.9854
11. Jinfang L, Yaoyao H, Tao Z, et al. Herbal verification of Xianmao in classical prescriptions. *Chin J Exp Trad Med Formulae*. 2024;30(14):199–208. doi:10.13422/j.cnki.syfjx.20240169
12. Wang Y, Li J, Li N. Phytochemistry and pharmacological activity of plants of Genus Curculigo: an updated review since 2013. *Molecules*. 2021;26(11). doi:10.3390/molecules26113396
13. Valls J, Richard T, Larronde F, et al. Two new benzylbenzoate glucosides from Curculigo orchioides. *Fitoterapia*. 2006;77(6):416–419. doi:10.1016/j.fitote.2006.05.013
14. Zuo A, Shen Y, Jiang Z, et al. Three new phenolic glycosides from Curculigo orchioides G. *Fitoterapia*. 2010;81(7):910–913. doi:10.1016/j.fitote.2010.06.003
15. Wang N, Zhao G, Zhang Y, et al. A network pharmacology approach to determine the active components and potential targets of Curculigo orchioides in the treatment of osteoporosis. *Med Sci Monit*. 2017;23:5113–5122. doi:10.12659/msm.904264
16. Zhuoyi AU, Yong ZHANG, Rongjie JIAN, Wenhan ZHU, Ran CHENG, Yanzhen WANG. Advances in the pharmacological mechanism of Xianmaooside. *Guangdong Chem*. 2023;50(05):117–119. doi:10.3969/j.issn.1007-1865.2023.05.037
17. Shen Q, Zeng D, Zhou Y, et al. Curculigoside promotes osteogenic differentiation of bone marrow stromal cells from ovariectomized rats. *J Pharm Pharmacol*. 2013;65(7):1005–1013. doi:10.1111/jphp.12054
18. Wang N, Li Z, Li S, et al. Curculigoside ameliorates bone loss by influencing mesenchymal stem cell fate in aging mice. *Front Cell Dev Biol*. 2021;9:767006. doi:10.3389/fcell.2021.767006
19. You W, Xu Z. Curculigoside promotes osteogenic differentiation of ADSCs to prevent ovariectomized-induced osteoporosis. *J Orthop Surg Res*. 2021;16(1):279. doi:10.1186/s13018-021-02389-3
20. Liu M, Li Y, Yang S. Curculigoside improves osteogenesis of human amniotic fluid-derived stem cells. *Stem Cells Dev*. 2014;23(2):146–154. doi:10.1089/scd.2013.0261
21. Ma C, Zhang J, Fu J, Cheng L, Zhao G, Gu Y. Up-regulation of VEGF by MC3T3-E1 cells treated with curculigoside. *Phytother Res*. 2011;25(6):922–926. doi:10.1002/ptr.3449
22. Wang Y, Zhao L, Wang Y, et al. Curculigoside isolated from Curculigo orchioides prevents hydrogen peroxide-induced dysfunction and oxidative damage in calvarial osteoblasts. *Acta Bioch Bioph Sin*. 2012;44(5):431–441. doi:10.1093/abbs/gms014
23. Bai HA, He YJ, Zhang Q, et al. Protective effect and mechanism of Xianmaooside against H2O2-induced oxidative damage in osteoblasts based on FoxO1 pathway. *Chin Med New Drugs Clin Pharmacol*. 2020;31(11):1296–1304. doi:10.19378/j.issn.1003-9783.2020.11.006
24. Zhang Q, Zhao L, Shen Y, et al. Curculigoside protects against excess-iron-induced bone loss by attenuating Akt-FoxO1-dependent oxidative damage to mice and osteoblastic MC3T3-E1 cells. *Oxid Med Cell Longev*. 2019;2019:9281481. doi:10.1155/2019/9281481
25. Fangbing ZHU, Yingliang ZHANG, Qiao HOU, et al. Effects and mechanism analysis of Xianmao glycoside on osteoblast proliferation and differentiation and expression of inflammatory factors. *Chin J Osteoporos*. 2019;25(05):642–648.
26. Liu M, Liu S, Zhang Q, et al. Curculigoside attenuates oxidative stress and osteoclastogenesis via modulating Nrf2/NF-kappaB signaling pathway in RAW264.7 cells. *J Ethnopharmacol*. 2021;275:114129. doi:10.1016/j.jep.2021.114129
27. Zhou X, Cao H, Guo J, Yuan Y, Ni G. Effects of BMSC-derived EVs on bone metabolism. *Pharmaceutics*. 2022;14(5):1012. doi:10.3390/pharmaceutics14051012
28. Da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci*. 2006;119(Pt 11):2204–2213. doi:10.1242/jcs.02932

29. Krasilnikova OA, Baranovskii DS, Lyundup AV, Shegay PV, Kaprin AD, Klabukov ID. Stem and somatic cell monotherapy for the treatment of diabetic foot ulcers: review of clinical studies and mechanisms of action. *Stem Cell Rev Rep*. 2022;18(6):1974–1985. doi:10.1007/s12015-022-10379-z
30. Zeng R, He J, Zhang Y, Liu X, Zhang Y, Tang Q. Experimental study on repairing skin defect by tissue-engineered skin substitute compositely constructed by adipose-derived stem cells and fibrin gel. *Eur Rev Med Pharmaco*. 2017;21:1–5.
31. El Agha E, Kramann R, Schneider RK, et al. Mesenchymal stem cells in fibrotic disease. *Cell Stem Cell*. 2017;21(2):166–177. doi:10.1016/j.stem.2017.07.011
32. Shi Y, Du L, Lin L, Wang Y. Tumour-associated mesenchymal stem/stromal cells: emerging therapeutic targets. *Nat Rev Drug Discov*. 2017;16(1):35–52. doi:10.1038/nrd.2016.193
33. Srouji S, Livne E. Bone marrow stem cells and biological scaffold for bone repair in aging and disease. *Mech Ageing Dev*. 2005;126(2):281–287. doi:10.1016/j.mad.2004.08.031
34. Tan F, Dai H. TAZ accelerates osteogenesis differentiation of mesenchymal stem cells via targeting PI3K/Akt. *Eur Rev Med Pharmaco*. 2019;23(3 Suppl):81–88. doi:10.26355/eurrev_201908_18633
35. Byun MR, Lee CH, Hwang J, et al. Phorbaketal A inhibits adipogenic differentiation through the suppression of PPARgamma-mediated gene transcription by TAZ. *Eur J Pharmacol*. 2013;718(1–3):181–187. doi:10.1016/j.ejphar.2013.08.035
36. Yu B, Huo L, Liu Y, et al. PGC-1alpha controls skeletal stem cell fate and bone-fat balance in osteoporosis and skeletal aging by inducing TAZ. *Cell Stem Cell*. 2018;23(4):615–623. doi:10.1016/j.stem.2018.09.001
37. Wang N, Xue P, Li Z, Li Y. IRS-1 increases TAZ expression and promotes osteogenic differentiation in rat bone marrow mesenchymal stem cells. *Biol Open*. 2018;7(12). doi:10.1242/bio.036194
38. Bacakova L, Zarubova J, Travnickova M, et al. Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review. *Biotechnol Adv*. 2018;36(4):1111–1126. doi:10.1016/j.biotechadv.2018.03.011
39. Bukowska J, Szostek-Mioduchowska AZ, Kopcewicz M, Walendzik K, Machcinska S, Gawronska-Kozak B. Adipose-derived stromal/stem cells from large animal models: from basic to applied science. *Stem Cell Rev Rep*. 2021;17(3):719–738. doi:10.1007/s12015-020-10049-y
40. Shafaei H, Kalarestaghi H. Adipose-derived stem cells: an appropriate selection for osteogenic differentiation. *J Cell Physiol*. 2020;235(11):8371–8386. doi:10.1002/jcp.29681
41. Lee J, Lee CY, Park J, et al. Differentiation of adipose-derived stem cells into functional chondrocytes by a small molecule that induces Sox9. *Exp Mol Med*. 2020;52(4):672–681. doi:10.1038/s12276-020-0424-y
42. Qin Y, Ge G, Yang P, et al. An update on adipose-derived stem cells for regenerative medicine: where challenge meets opportunity. *Adv Sci*. 2023;10(20):e2207334. doi:10.1002/advs.202207334
43. Ye C, Zhang W, Hang K, et al. Extracellular IL-37 promotes osteogenic differentiation of human bone marrow mesenchymal stem cells via activation of the PI3K/AKT signaling pathway. *Cell Death Dis*. 2019;10(10):753. doi:10.1038/s41419-019-1904-7
44. An Y, Zhao J, Nie F, Wu Y, Xia Y, Li D. Parathyroid hormone (PTH) promotes ADSC osteogenesis by regulating SIK2 and Wnt4. *Biochem Biophys Res Commun*. 2019;516(2):551–557. doi:10.1016/j.bbrc.2019.06.084
45. Yang X, Song Y, Li L, Liu D, Chen G. miR-1249-5p regulates the osteogenic differentiation of ADSCs by targeting PDX1. *J Orthop Surg Res*. 2021;16(1):10. doi:10.1186/s13018-020-02147-x
46. De Coppi P, Bartsch GJ, Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol*. 2007;25(1):100–106. doi:10.1038/nbt1274
47. Yeh Y, Wei H, Lee W, et al. Cellular cardiomyoplasty with human amniotic fluid stem cells: in vitro and in vivo studies. *Tissue Eng Pt A*. 2010;16(6):1925–1936. doi:10.1089/ten.TEA.2009.0728
48. Liu Y, Wu J, Zhu Y, Han J. Therapeutic application of mesenchymal stem cells in bone and joint diseases. *Clin Exp Med*. 2014;14(1):13–24. doi:10.1007/s10238-012-0218-1
49. Yoon BS, Moon J, Jun EK, et al. Secretory profiles and wound healing effects of human amniotic fluid-derived mesenchymal stem cells. *Stem Cells Dev*. 2010;19(6):887–902. doi:10.1089/scd.2009.0138
50. Trohatou O, Anagnou NP, Roubelakis MG. Human amniotic fluid stem cells as an attractive tool for clinical applications. *Curr Stem Cell Res T*. 2013;8(2):125–132. doi:10.2174/1574888X11308020003
51. Huang W, Yang S, Shao J, Li Y. Signaling and transcriptional regulation in osteoblast commitment and differentiation. *Front Biosci*. 2007;12(8–12):3068–3092. doi:10.2741/2296
52. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. *Gene*. 2004;341:19–39. doi:10.1016/j.gene.2004.06.044
53. Hu H, Hilton MJ, Tu X, Yu K, Ornitz DM, Long F. Sequential roles of Hedgehog and Wnt signaling in osteoblast development. *Development*. 2005;132(1):49–60. doi:10.1242/dev.01564
54. Neve A, Corrado A, Cantatore FP. Osteocalcin: skeletal and extra-skeletal effects. *J Cell Physiol*. 2013;228(6):1149–1153. doi:10.1002/jcp.24278
55. Langdahl B, Ferrari S, Dempster DW. Bone modeling and remodeling: potential as therapeutic targets for the treatment of osteoporosis. *Ther Adv Musculoskel*. 2016;8(6):225–235. doi:10.1177/1759720X16670154
56. Yin W, Qizhen S, Liyan M. Proliferation, differentiation and calcification of MC3T3-E1 osteoblasts by Xianmaooside. *World Sci Technol*. 2011;13(05):852–855. doi:10.11842/wst.2011.5
57. Street J, Bao M, Deguzman L, et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc Natl Acad Sci U S A*. 2002;99(15):9656–9661. doi:10.1073/pnas.152324099
58. Otomo H, Sakai A, Uchida S, et al. Flt-1 tyrosine kinase-deficient homozygous mice result in decreased trabecular bone volume with reduced osteogenic potential. *Bone*. 2007;40(6):1494–1501. doi:10.1016/j.bone.2007.02.007
59. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J*. 1999;13(1):9–22. doi:10.1096/fasebj.13.1.9
60. Basu S, Michaelsson K, Olofsson H, Johansson S, Melhus H. Association between oxidative stress and bone mineral density. *Biochem Biophys Res Commun*. 2001;288(1):275–279. doi:10.1006/bbrc.2001.5747

61. Bai X, Lu D, Bai J, et al. Oxidative stress inhibits osteoblastic differentiation of bone cells by ERK and NF-kappaB. *Biochem Biophys Res Commun*. 2004;314(1):197–207. doi:10.1016/j.bbrc.2003.12.073
62. Kousteni S. FoxO1: a molecule for all seasons. *J Bone Miner Res*. 2011;26(5):912–917. doi:10.1002/jbmr.306
63. Ono T, Nakashima T. Recent advances in osteoclast biology. *Histochem Cell Biol*. 2018;149(4):325–341. doi:10.1007/s00418-018-1636-2
64. Kim HS, Nam ST, Mun SH, et al. DJ-1 controls bone homeostasis through the regulation of osteoclast differentiation. *Nat Commun*. 2017;8 (1):1519. doi:10.1038/s41467-017-01527-y
65. Park JH, Lee NK, Lee SY. Current understanding of RANK signaling in osteoclast differentiation and maturation. *Mol Cells*. 2017;40 (10):706–713. doi:10.14348/molcells.2017.0225
66. Ahn YJ, Lim JW, Kim H. Docosahexaenoic acid induces expression of NAD(P)H: quinone oxidoreductase and heme oxygenase-1 through activation of Nrf2 in cerulein-stimulated pancreatic acinar cells. *Antioxidants-Basel*. 2020;9(11). doi:10.3390/antiox9111084
67. Qinjian WANG, Pan ZHANG, Ruixin ZHANG, et al. Effects of Xianmaooside regulating lncRNA KCNQ1OT1/miR-214-5p axis on bone metabolism in osteoporotic rats by Qinjian Wang, Pan Zhang, Ruixin Zhang, and Popo Li. *Chin J Osteoporos*. 2024;30(09):1261–1267.
68. Wang QJ, Zhang RX, Zhang P, et al. Effect of cynarin regulating lncRNA MEG3/miR-181a-5p pathway on autophagy in osteoporotic rat osteoblasts. *Chin J Osteoporos*. 2023;29(10):1409–1414+1465. doi:10.3969/j.issn.1006-7108.2023.10.002
69. Ruyu HAN, Yutong LI, Yangyang LI, et al. Animal experiments on the modulation of osteoblast differentiation by sennosides in the treatment of osteoporosis. *Chin J Stomatol*. 2019;54(9):632–638. doi:10.3760/cma.j.issn.1002-0098.2019.09.010
70. Zhao L, Liu S, Wang Y, et al. Effects of Curculigoside on memory impairment and bone loss via anti-oxidative character in APP/PS1 mutated transgenic mice. *PLoS One*. 2015;10(7):e0133289. doi:10.1371/journal.pone.0133289
71. Zhu F, Wang J, Ni Y, et al. Curculigoside protects against Titanium particle-induced osteolysis through the enhancement of osteoblast differentiation and reduction of osteoclast formation. *J Immunol Res*. 2021;2021:5707242. doi:10.1155/2021/5707242
72. Yang J, Peng W, Zhang M. LncRNA KCNQ1OT1 promotes osteogenic differentiation via miR-205-5p/RICTOR axis. *Exp Cell Res*. 2022;415 (1):113119. doi:10.1016/j.yexcr.2022.113119
73. Wu P, Ji ZW, Wang ZX, et al. Effect of miR-214-5p on osteoblastic differentiation and osteoporosis by targeting and regulating PTEN expression. *Armed Forces Med*. 2022;33(05):431–436. doi:10.14010/j.cnki.wjyx.2022.05.024
74. Wang Q, Li Y, Zhang Y, et al. LncRNA MEG3 inhibited osteogenic differentiation of bone marrow mesenchymal stem cells from postmenopausal osteoporosis by targeting miR-133a-3p. *Biomed Pharmacother*. 2017;89:1178–1186. doi:10.1016/j.biopha.2017.02.090
75. Long Z, Dou P, Cai W, Mao M, Wu R. MiR-181a-5p promotes osteogenesis by targeting BMP3. *Aging*. 2023;15(3):734–747. doi:10.18632/aging.204505
76. Jiang Y, Wang Z, Liu X, et al. The protective effects of osteocyte-derived extracellular vesicles against Alzheimer's disease diminished with aging. *Adv Sci*. 2022;9(17):e2105316. doi:10.1002/advs.202105316
77. Lao KJ, Chen JL, Huang MY, et al. Advances in anti-Alzheimer's disease drugs based on the Aβ doctrine. *J Food Biotechnol*. 2023;42 (08):1–10.
78. Li S, Liu B, Zhang L, Rong L. Amyloid beta peptide is elevated in osteoporotic bone tissues and enhances osteoclast function. *Bone*. 2014;61:164–175. doi:10.1016/j.bone.2014.01.010
79. Kwan Tat S, Padrines M, Theoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. *Cytokine Growth F R*. 2004;15(1):49–60. doi:10.1016/j.cytogfr.2003.10.005
80. Learmonth ID, Young C, Rorabeck C. The operation of the century: total hip replacement. *Lancet*. 2007;370(9597):1508–1519. doi:10.1016/S0140-6736(07)60457-7
81. Prkic A, Welsink C, The B, van den Bekerom MPJ, Eygendaal D. Why does total elbow arthroplasty fail today? A systematic review of recent literature. *Arch Orthop Traum Su*. 2017;137(6):761–769. doi:10.1007/s00402-017-2687-x
82. Fehring TK, Odum S, Griffin WL, Mason JB, Nadaud M. Early failures in total knee arthroplasty. *Clin Orthop Relat R*. 2001;392 (392):315–318. doi:10.1097/00003086-200111000-00041
83. Zhao Y, Wang HL, Li TT, Yang F, Tzeng CM. Baicalin ameliorates dexamethasone-induced osteoporosis by regulation of the RANK/RANKL/OPG signaling pathway. *Drug Des Devel Ther*. 2020;14:195–206. doi:10.2147/DDDT.S225516
84. Nakashima T, Hayashi M, Fukunaga T, et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat Med*. 2011;17(10):1231–1234. doi:10.1038/nm.2452
85. Indridason OS, Franzson L, Sigurdsson G. Serum osteoprotegerin and its relationship with bone mineral density and markers of bone turnover. *Osteoporos Int*. 2005;16(4):417–423. doi:10.1007/s00198-004-1699-x
86. Taranta A, Fortunati D, Longo M, et al. Imbalance of osteoclastogenesis-regulating factors in patients with celiac disease. *J Bone Miner Res*. 2004;19(7):1112–1121. doi:10.1359/JBMR.040319
87. Burke JE. Structural basis for regulation of phosphoinositide kinases and their involvement in human disease. *Mol Cell*. 2018;71(5):653–673. doi:10.1016/j.molcel.2018.08.005
88. Mayer IA, Arteaga CL. The PI3K/AKT pathway as a target for cancer treatment. *Annu Rev Med*. 2016;67(1):11–28. doi:10.1146/annurev-med-062913-051343
89. Gu YX, Du J, Si MS, Mo JJ, Qiao SC, Lai HC. The roles of PI3K/Akt signaling pathway in regulating MC3T3-E1 preosteoblast proliferation and differentiation on SLA and SLActive titanium surfaces. *J Biomed Mater Res A*. 2013;101(3):748–754. doi:10.1002/jbm.a.34377
90. Zou W, Yang S, Zhang T, et al. Hypoxia enhances glucocorticoid-induced apoptosis and cell cycle arrest via the PI3K/Akt signaling pathway in osteoblastic cells. *J Bone Miner Metab*. 2015;33(6):615–624. doi:10.1007/s00774-014-0627-1
91. Wang Z, Li Z, Ji H. Direct targeting of 尾-catenin in the Wnt signaling pathway: current progress and perspectives. *Med Res Rev*. 2021;41 (4):2109–2129. doi:10.1002/med.21787
92. Kim EK, Choi E. Compromised MAPK signaling in human diseases: an update. *Arch Toxicol*. 2015;89(6):867–882. doi:10.1007/s00204-015-1472-2
93. Xu Z, Sun J, Tong Q, et al. The role of ERK1/2 in the development of diabetic cardiomyopathy. *Int J Mol Sci*. 2016;17(12):2001. doi:10.3390/ijms17122001
94. Li Y, Ge C, Franceschi RT. MAP kinase-dependent RUNX2 phosphorylation is necessary for epigenetic modification of chromatin during osteoblast differentiation. *J Cell Physiol*. 2017;232(9):2427–2435. doi:10.1002/jcp.25517

95. Yuan T, Xu H, Zhao L, et al. Pharmacokinetic and tissue distribution profile of curculigoside after oral and intravenously injection administration in rats by liquid chromatography-mass spectrometry. *Fitoterapia*. 2015;101:64–72. doi:10.1016/j.fitote.2014.12.012
96. Zhao G, Yuan F, Zhu J. An LC-MS/MS method for determination of curculigoside with anti-osteoporotic activity in rat plasma and application to a pharmacokinetic study. *Biomed Chromatogr*. 2014;28(3):341–347. doi:10.1002/bmc.3025
97. Choi J, Li X. The effect of verapamil on the pharmacokinetics of paclitaxel in rats. *Eur J Pharm Sci*. 2005;24(1):95–100. doi:10.1016/j.ejps.2004.10.002
98. Wang K, Zhao J, Lang J. The effects of verapamil on the pharmacokinetics of curculigoside in rats. *Pharm Biol*. 2016;54(12):3001–3008. doi:10.1080/13880209.2016.1199043
99. Weng Y, Yuan X, Fan S, et al. 3D-printed biomimetic hydroxyapatite composite scaffold loaded with curculigoside for rat cranial defect repair. *Acs Omega*. 2024;9(24):26097–26111. doi:10.1021/acsomega.4c01533
100. Zhuo Y, Li M, Jiang Q, et al. Evolving roles of natural terpenoids from traditional Chinese medicine in the treatment of osteoporosis. *Front Endocrinol*. 2022;13:901545. doi:10.3389/fendo.2022.901545
101. Xu D, Wu L, Chen Q. Editorial: bone and cartilage diseases-the role and potential of natural products. *Front Pharmacol*. 2022;13:938303. doi:10.3389/fphar.2022.938303
102. Chen Y, Jia L, Han T, et al. Osteoporosis treatment: current drugs and future developments. *Front Pharmacol*. 2024;15:1456796. doi:10.3389/fphar.2024.1456796