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Review Article

Research progress on the role of *Epimedium* in chronic bone and joint diseases

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Abstract: Chronic bone and joint diseases severely affect the functional status of bone, cartilage, and soft tissues, thus leading to pain and dysfunction in the affected areas. Patients with these diseases can be treated by conservative methods such as oral medications in the early stages, while surgical interventions are commonly required for those in the advanced stage. As conventional anti-osteoporosis (OP) drugs, anti-inflammatory drugs, painkillers, and immunomodulators may induce side effects and drug resistance in the treatment process, it is necessary to explore new therapeutic drugs for the adjuvant treatment of chronic bone and joint diseases at an early or advanced stage. *Epimedium* is an herbal medicine in traditional Chinese medicine (TCM) and has been used for more than 2,000 years. As the main active ingredient in this herbal medicine, icariin (ICA) is effective in the treatment of chronic bone and joint diseases. This ingredient can be involved in the pathophysiological process of these diseases *via* modulating the osteogenic and chondrogenic differentiation of bone marrow mesenchymal stem cells (BMSCs), inhibiting osteoclastogenesis, resisting inflammation, protecting the extracellular matrix (ECM), regulating oxidative stress (OS), and participating in immunomodulation. In this study, the role of *Epimedium* in the prevention and treatment of chronic bone and joint diseases was systematically reviewed. The results demonstrated that *Epimedium* can be used as a complementary and alternative medicine for the treatment of chronic bone and joint diseases. In this paper, the mechanism of action and potential application of *Epimedium* in the prevention and treatment of chronic bone and joint diseases were summarized. These findings may lay a foundation for the further development and clinical application of *Epimedium* as a medicinal food source.

Keywords: *Epimedium*; icariin; chronic bone and joint diseases; bone marrow mesenchymal stem cells (BMSCs)

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1 Introduction

Common chronic bone and joint diseases in the field of orthopedics include osteoporosis (OP), osteoarthritis (OA), rheumatoid arthritis (RA), and intervertebral disc disease (IVDD). These diseases are mainly manifested as pain and motor dysfunction[1-5]. In the context of population aging, the incidence of chronic bone and joint diseases has gradually increased in China, which has imposed a heavy burden on patients' families and social healthcare[6-8]. Since the pathogenesis of chronic bone and joint diseases has not been fully clarified, there is a lack of effective interventions for them. Patients with these diseases can be treated by conservative methods such as oral medications in the early stage, while surgical interventions are required for those in the advanced stage. Therefore, early intervention of chronic bone and joint diseases based on medicinal and food-derived substances contributes to delaying the progression of these diseases[9-12].

Epimedium is designated based on the phenomenon that goats can boost the number of mating sessions after eating this plant. It is also known as the "Three Branches" due to the three branches on its stem, each with three leaves. *Epimedium* is categorized into the Berberidaceae family, and there are 68 *Epimedium* species worldwide, 58 of which are found in China. As an herbal medicine in traditional Chinese medicine (TCM), *Epimedium* has been used

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in China for more than 2,000 years. *Epimedium* was first mentioned in *Shen Nong Ben Cao Jing* (*Divine Farmer's Classic of Materia Medica*), and it is classified as a medium in *Ben Cao Gang Mu* (*Compendium of Materia Medica*). As recorded in the classic work, this herbal medicine has been used for the treatment of sexual dysfunction, OP, irregular menstruation, asthma, cancer, OA, and RA, and it has also been used as a food-based health product[13] *Epimedium* contains 141 flavonoids, 31 lignins, 12 ionones, 9 phenol glycosides, 6 phenylethanoid glycosides, and 5 sesquiterpenes[14]. Among them, icariin (ICA) is the main active ingredient of *Epimedium*, accounting for 6.5% of the dry weight of the ethanolic extract. As an isoprenoid flavonoid (accounting for more than 50% of the weight of the ethanolic extract), ICA has been used as a marker for the standardization of the quality of *Epimedium* extracts[15]. It has been corroborated that ICA can regulate and participate in the pathogenesis and progression of skeletal degenerative diseases through a variety of mechanisms. Besides, it also plays a role in resolving OP, promoting articular cartilage regeneration and repair, regulating RA, and alleviating degenerative disc lesions. However, the acting mechanism of ICA has not been fully clarified, and it would be of great significance to generalize its role in the treatment of chronic bone and joint diseases. Given these facts, the pathophysiological mechanisms of ICA in the treatment of degenerative bone diseases were summarized in this study. These findings may lay a foundation for the development and clinical application of *Epimedium* as a medicinal substance.

2 The role of ICA in OP

Osteoporosis is a systemic metabolic bone disease characterized by excessive bone loss, decreased bone density, bone microstructure injury, and increased fat in the bone marrow cavity[16]. As reported in epidemiologic surveys, the prevalence of OP is 6.46% in men and 29.13% in women among Chinese individuals aged over 50 years[17]. Osteoporosis fracture (OF), also known as fragility fracture, is one of the serious consequences of OP. Patients with OF may suffer from decreased bone strength and increased fragility, and they may experience fractures when subjected to low-energy external forces during daily activities[18]. These factures usually affect the spine, hip, distal radius, and proximal humerus[19,20]. After the occurrence of osteoporotic hip fracture, the cumulative mortality of elderly patients is close to 70%, and those surviving this fracture will suffer from various complications, which may significantly affect their quality of life[21]. As the aging society in China intensifies, 4.83 million individuals will suffer from osteoporotic fractures by 2035[22]. A clinical study in healthy postmenopausal women (58 patients in total, aged (57.9 ± 8.9) years) taking *Epimedium* spp (EP) or placebo in a double-blind, randomised fashion showed that at weeks 3 and 6 of continuous dosing and 2 weeks after cessation of dosing, the level of epimedium glycosides in the blood of patients at week 6 was (11.5 ± 2.3) nmol/L, whereas after discontinuation the presence of epimedium glycosides in the blood was undetectable. The presence of ICA was not detected in the blood after discontinuation, and the elevation of Icariin in the blood was closely related to the high expression of bone-specific alkaline phosphatase[23]. In another clinical follow-up study, 155 postmenopausal women (47–70 years old) who met the study requirements were divided into groups containing *Epimedium*. In another clinical follow-up study, 155 postmenopausal women (47–70 years old) who met the study criteria were divided into a prescription group containing *Epimedium* and a placebo group over a period of 5 years; no significant adverse drug reactions were observed in either group. Bone mineral density (BMD) increased significantly in the treatment group and decreased significantly in the control group after 5 years; the incidence of fractures was 2.4 times lower in the treatment group than in the control group[24]. In the treatment of osteoporotic bone repair, bone-injured rats were treated with 150 mg/kg of ICA by gavage and 20 μg/kg of teriparatide by subcutaneous injection daily for 8 weeks, and both ICA and teriparatide promoted osteoporotic bone healing and at the same time upregulated the expression of bone morphogenetic protein 2 (BMP2) at the site of bone repair, and the results of the treatments showed no significant difference[25]. As suggested in existing studies, ICA plays a role in promoting the osteogenic differentiation of BMSCs, regulating osteogenic-lipogenic differentiation, and inhibiting osteogenic differentiation (Fig. 1), and it is one of the optional substances for the treatment of OP.

2.1 The regulation of BMSCs by ICA

Bone marrow mesenchymal stem cells are capable of self-renewal and multidirectional differentiation, which are essential for bone regeneration and repair[26]. It has been demonstrated that ICA can promote the osteogenic differentiation of rat-derived BMSCs by regulating the Wnt/β-catenin and PI3K/AKT signaling pathway and up-regulating the expression of BMP2 and Runt-related transcription factor 2 (RUNX2) (Fig. 2)[27,28]. The effect of BMSCs in the treatment of OP is closely related to their migration ability, and the impaired migration ability of BMSCs in OP may result in less aggregation of BMSCs at the affected site, thus decreasing their osteogenic ability[29]. Feng *et al.*[30] applied ICA to rabbit-derived BMSCs and found that ICA could promote the proliferation and migration of BMSCs through the mitogen-activated protein kinase (MAPK) signaling pathway, which enabled BMSCs to reach the lesion site faster. It has been shown that ICA can restore the activity of BMSCs under OS, enhance collagen secretion and extracellular matrix mineralization (ECMM), up-regulate the expression of osteogenesis-related genes (*BMP2*, *RUNX2*, collagen type I (*COL I*), alkaline phosphatase (*ALP*), and osteocalcin (*OCN*)), and inhibit macrophage inflammation at the same time, thus fulfilling functions in the treatment of OP[31]. The bone-enhancing effect of ICA at the cellular level can be verified based on the experiment using human BMSCs (hBMSCs), which is closer to clinical practice. Fan *et al.*[32] extracted BMSCs from the bone marrow of volunteers, and they applied different concentrations of ICA to hBMSCs to reveal their proliferation and osteogenic differentiation ability. The experimental results showed that the ICA at a concentration from 1×10^{-9} to 1×10^{-6} mol/L could significantly promote the proliferation and osteogenic differentiation of hBMSCs in a concentration-dependent manner. It can be seen that ICA can promote the proliferation and osteogenic differentiation of BMSCs, but the specific regulatory mechanism needs to be further investigated. Some scholars reported that adding ICA to the diet of osteoporotic rats for 12 weeks significantly increased the BMD of their lumbar vertebra and femur. Besides, the biomechanical strength of their lumbar vertebra and femur increased, and the structure of bone trabeculae was restored. The quantitative polymerase chain reaction (qPCR) assay of femoral samples indicated that ICA could treat OP by regulating the Wnt/β-catenin signaling pathway and activating the expression of the downstream *RUNX2* gene[33]. Xia *et al.*[34]

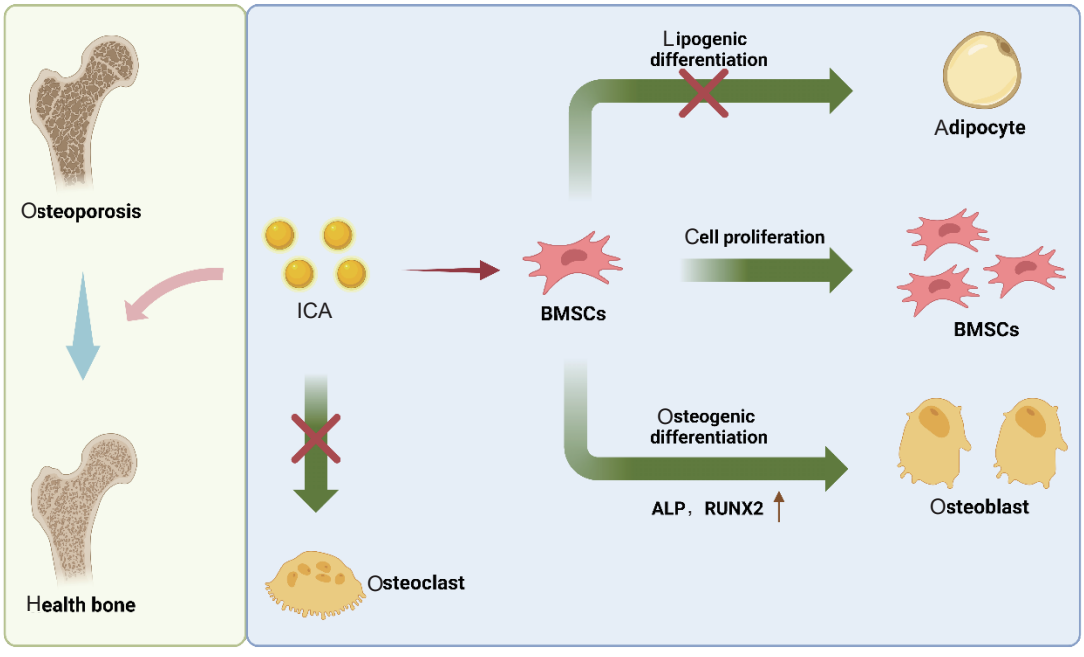

Figure 1 Role of ICA in promoting the osteogenic differentiation of BMSCs, regulating osteogenic-lipogenic differentiation, and inhibiting osteoclastogenesis. Figures created with Biorender.com.

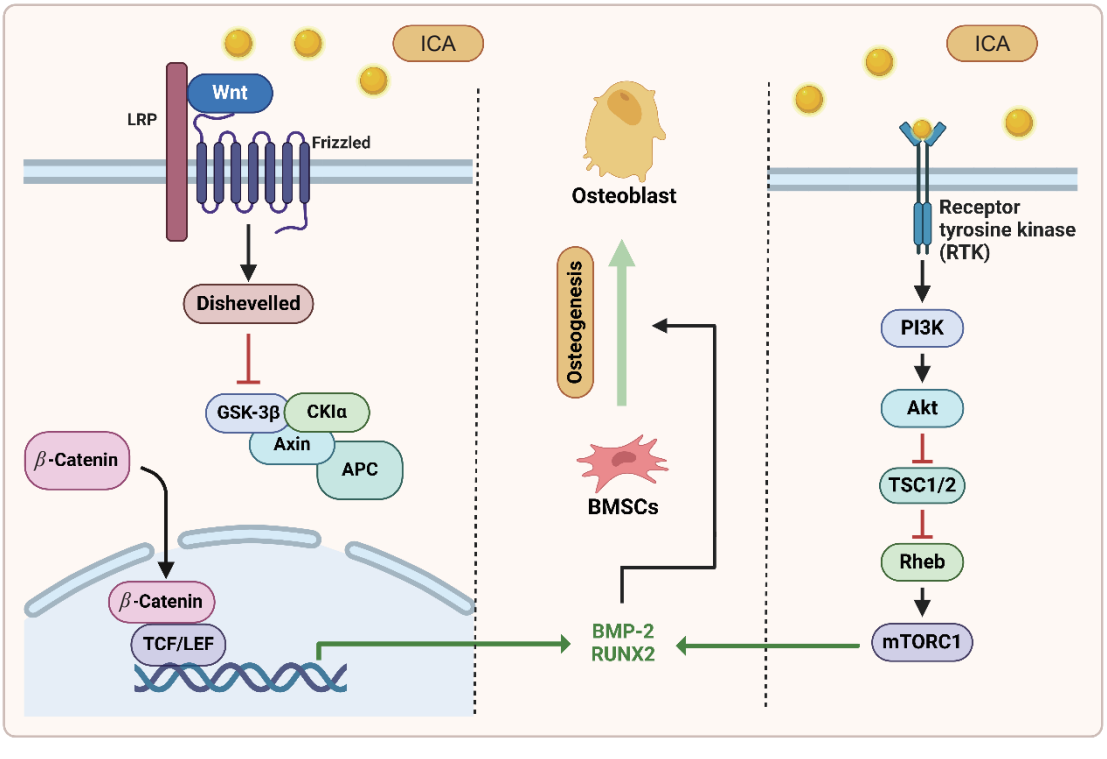

Figure 2 ICA promotes osteogenic differentiation of BMSCs by regulating Wnt/β-catenin and PI3K/AKT signalling pathway. Figures created with Biorender.com.

screened six differentially expressed genes (DEGs) related to proliferation and osteogenesis through the RNA sequencing analysis of ICA-activated hBMSCs. They found that the most obvious difference was in GLI-1, and knockdown of GLI-1 attenuated the proliferation and osteogenesis of ICA in BMSC. Moreover, the osteogenic-lipogenic differentiation of BMSCs was unbalanced in OP, with the lipogenic differentiation taking a dominant position, whereas ICA could inhibit the lipogenic differentiation of BMSCs and improve OP by suppressing the expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) and CCAAT-enhancer-binding proteins (C/EBPs)[35]. In recent years, the development of bone tissue engineering technology has provided a new strategy for bone regeneration and repair. After ICA and BMSCs were co-loaded onto hydrogel and the composite was implanted into the bone defects of the distal femur of rats, the indexes related to the bone volume fraction (BV/TV) and BMD in the defect area were significantly improved compared with those of the blank group. Additionally, the

histological sections revealed that there were more newborn bone tissues at the defect, which significantly accelerated bone defect repair[36]. After the calcium phosphate bone cement scaffolds were loaded with ICA and implanted into the cranial defects of osteoporotic rats for 8 weeks, the CPC/ICA scaffold group had a significantly increased area of new bones and more neovascularization at the bone defects. This result suggested that ICA released from the CPC scaffolds exerted a local pro-bone repair and angiogenesis role[37]. In OP, the osteogenic differentiation ability of senescent BMSCs is impaired, and ICA can directly act on BMSCs to promote their proliferation, migration, and osteogenic differentiation, which significantly promotes osteoporotic bone healing at the animal level.

2.2 The regulation of OBs by ICA

Bone remodeling is a dynamic physiological process in the skeletal system that includes bone formation by osteoblasts (OBs) and bone resorption by osteoclasts (OCs). Among them, OBs are differentiated from BMSCs and located on the bone surface, and they are responsible for synthesizing and secreting bone matrix and promoting bone mineralization[38]. The differentiation ability of BMSCs into OBs decreases with an increase in age, along with a decrease in the activity of the OBs, which can lead to a slower rate of new bone formation[39,40]. Therefore, regulating the activity and differentiation ability of OBs and enhancing their bone formation efficiency are important for the treatment of OP and osteoporotic fractures. Sun *et al.*[41] found that 50 ng/mL ICA augmented the activities of ALP, osteocalcin, and Col I in hFOB1.19 human OBs and increased the number of calcified nodules. In the primary OBs derived from rats, Ma *et al.*[42] found that 1×10^{-5} mol/L ICA significantly elevated the activity of ALP in OBs, increased the production of calcified nodules, and up-regulated the protein expression of the OB transcription factors (osterix (Osx), RUNX2, and COL I). It has also been demonstrated that 1×10^{-5} mol/L ICA is the optimal concentration to promote the proliferation and osteogenic differentiation of OBs. Under this concentration, ICA can significantly up-regulate the mRNA expression of *Runx-2* and *Osx*, both of which are key transcription factors for OB differentiation and bone formation[43]. Wang *et al.*[44] applied ICA to OBs and found that ICA significantly promoted the proliferation and ALP activity of OBs. Further, it also up-regulated the gene expression of ALP and RUNX2 through the Wnt/β-catenin signaling pathway, thus promoting the proliferation and differentiation of OBs. Ferroptosis and the accumulation of reactive oxygen species (ROS) can lead to the generation of OS and inhibit the activity of OBs. Xiang *et al.*[45] confirmed that ICA could significantly reduce the level of ROS in OBs, increase the protein expression level of glutathione peroxidase 4 (GPX4) and solute carrier family 7 member 11 (SLC7A11), inhibit the occurrence of ferroptosis in OBs by activating the antioxidant Nrf2/HO-1 signaling pathway, and simultaneously up-regulate the protein expression levels of GPX4, Nrf2, and Runx2 at the fracture site, thus accelerating the OF healing in rats. MC3T3-E1 cells are a category of mouse embryonic OB precursor cells, and 1 μmol/L ICA can increase the mRNA expression levels of *Runx2*, *ALP*, *BMP2*, and osteopontin (*OPN*) in MC3T3-E1 cells through the miR-153/Runx2 pathway, and enhance the osteogenic differentiation of MC3T3-E1 cells[46]. In addition, ICA can promote the proliferation of OBs and reduce the apoptosis of OCs by activating the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and MAPK pathways. Moreover, ICA can significantly up-regulate the mRNA expression of *Col* I and *ALP* in a dose-dependent manner[47]. The osteoconductive effects of BMP2 have been established. To further validate the role of ICA in osteogenesis, Xie *et al.*[48] compared BMP2 and ICA. The results indicated that ICA at a concentration of 30 μg/mL and BMP2 at a concentration of 50 μg/mL were comparable in promoting the proliferation of OBs and up-regulating the expression of osteogenesis-related genes (ALP and osteoprotegerin (OPG)). Then, ICA and BMP2 were loaded onto nano-hydroxyapatite/collagen/polylactic acid scaffolds, which were implanted into the bone defect sites of rabbits. The results revealed that both could achieve favorable repair effects, without significant differences in the repair effects after the treatment for 8 weeks.

2.3 The regulation of OCs by ICA

As the precursors of OCs, peripheral blood mononuclear cells are located in the bone marrow, while mature OCs are located on the surface of the bone and constitute a category of multinucleated cells responsible for bone resorption[49]. The OPG/RANKL/RANK signaling pathway plays an important role in the differentiation and maturation of OBs. The receptor activator of nuclear factor-kappa B (RANK) is expressed in OB progenitors and mature OBs. The receptor activator of nuclear factor kappa B ligand (RANKL) is also known as the OB differentiation factor (ODF) and osteoprotegerin ligand (OPGL). RANKL is highly expressed on the membrane of OBs, and it usually binds to RANK on the surface of OB precursors. This further activates the family of TNF receptor-associated factors (TRAFs), which promotes the activation and differentiation of OCs and inhibits their apoptosis[50,51]. OPG can inhibit OC formation by blocking the binding of RANKL to RANK. Meanwhile, the expression of RANK in bone marrow precursors is also regulated by macrophage colony-stimulating factors (M-CSFs)[52]. Thus, OCs are involved in the development of almost all bone loss-related diseases by regulating the OPG/RANKL/RANK signaling pathway[53]. In recent years, experiments on the regulation of the activity and generation of OCs by ICA have been gradually reported. Chen *et al.*[54] demonstrated that ICA inhibited RANKL-induced osteoclast differentiation, as evidenced by a reduction in the number of positive cells was observed in tartrate-resistant acid phosphatase (TRAP) staining. This may be attributed to the up-regulated mRNA expression of OPG and down-regulated mRNA expression of RANKL under the action of ICA[55]. NFATc1 is a major transcription factor for osteoclast, and it has been confirmed that ICA can directly inhibit TRAF6, thereby blocking the RANKL-TRAF6-induced differentiation of OCs, inhibiting the activation of ERK1/2 and nuclear factor kappa B (NF-κB), down-regulating the expression of RANK, TRAP, and NFATc1, and decreasing osteoclast[56]. Meanwhile, ICA can inhibit OC differentiation and bone resorption by inhibiting the MAPK/NF-κB signaling pathway and suppressing the protein expression of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and hypoxia-inducible factor 1α (HIF-1α)[57]. ICA inhibits ROS generation by activating Nrf2 signaling *via* targeting Cullin 3, reduces osteoclast, reconstructs bone trabecular microstructures, and alleviates bone loss in OVX rats[58]. With an increase in the age of mice, the intracellular adenosine triphosphate (ATP) level in OCs decreased and bone resorption activities increased. However, ICA increased the intracellular ATP level in OCs, suggesting that ICA can regulate the energy metabolism level of OCs and then decrease the bone resorption capacity of OCs[59]. After being loaded with ICA,

hydroxyapatite/alginate (HAA) porous composite scaffolds were implanted into rabbit radial defects, and TRAP staining at the bone repair site showed a significant decrease in the number of OCs[60]. Zhang *et al.*[61] constructed a co-culture system using MC3T3-E1 cells and RAW264.7 cells in Transwell chambers to investigate the effect of ICA on the co-culture system. The experimental results showed that ICA up-regulated the mRNA expression of *OPG* in MC3T3-E1 cells in the co-culture system and down-regulated that of RANK and NF-κB in RAW264.7 cells.

In summary, after the occurrence of OP, the stem cell viability and osteogenic differentiation capacity decrease, and osteogenic-lipogenic differentiation is unbalanced. The results of the above experiments indicate that ICA can directly regulate the functional status of BMSCs, promote their proliferation and osteogenic differentiation, and contribute to bone formation. Meanwhile, ICA can inhibit the activity of OCs while activating OBs and reduce bone resorption. ICA exerts a bidirectional regulatory effect on bone formation and bone resorption, thus exhibiting great application potential in the treatment of OP (Fig. 3).

3 The role of ICA in OA

Osteoarthritis is a chronic degenerative disease characterized by articular cartilage degeneration, synovial tissue proliferation, and inflammatory responses. It is one of the leading causes of disability in patients[62]. Since 1950, the number of patients with OA in the knee in the United States has increased by a factor of 2.1, and as of today, more than 500 million people worldwide suffer from OA[63]. Due to the lack of proper clinical management of patients in the early stages of the disease, arthroplasty is often selected as the primary treatment for patients with OA at an advanced stage, and the number of patients undergoing arthroplasty is increasing at a rate of 10% per year[64]. Therefore, a timely clinical intervention at an early stage of OA is of great significance for the prevention and treatment of this disease. ICA can promote the proliferation and differentiation of chondrocytes, enhance anti-inflammatory effects, prevent cartilage degradation, and promote cartilage repair[65], making it a promising herbal substance for the early treatment of OA (Fig. 4).

3.1 The regulation of inflammation in OA by ICA

Macrophages are categorized into classically activated macrophages (M1) and alternatively activated macrophages (M2), which play an important role in OA[66]. Among them, such pro-inflammatory mediators as lipopolysaccharide (LPS) and interferon-γ (IFN-γ) can induce macrophage M1 polarization, which leads to the secretion of inflammatory cytokines, such as interleukins (IL-6 and IL-1β), inducible nitric oxide synthase (iNOS), and TNF-α, thus exerting a pro-inflammatory effect. In contrast, macrophage M2 polarization is induced by IL-4 and IL-13, which can secrete IL-10 and arginase, further blocking the activity of IL-1β and iNOS and exerting anti-inflammatory effects[67-69]. It has been demonstrated that ICA significantly reduces the expression levels of IL-1β and IL-6 in inflammatory responses[70]. Inhibiting the M1 polarization of macrophages exerts anti-inflammatory effects[71]. Meanwhile, ICA can induce the M2 polarization of macrophages to exert anti-inflammatory and tissue regenerative repair effects[72]. The inflammatory response in OA is a complex and variable pathophysiological phenomenon, and untimely resolved and uncontrolled inflammation is destructive and underlies chronic inflammation[73]. Therefore, eliminating the inflammatory response in OA and preventing its further damage to the synovium, cartilage, and other tissues are of great clinical significance. ICA can down-regulate the protein expression of IL-6, IL-8, and TNF-α, inhibit the inflammatory response, and protect chondrocytes from the further erosion of inflammatory factors in the OA cell model[74]. Yu *et al.*[75] designed ICA/tannic acid-nanodiamonds (ICA/TA-NDs) and injected them into the knee joints of OA rats. The results showed that ICA/TA-NDs inhibited the mRNA expression of such pro-inflammatory factors as matrix metalloproteinase-3 (*MMP*-3), cyclooxygenase-2

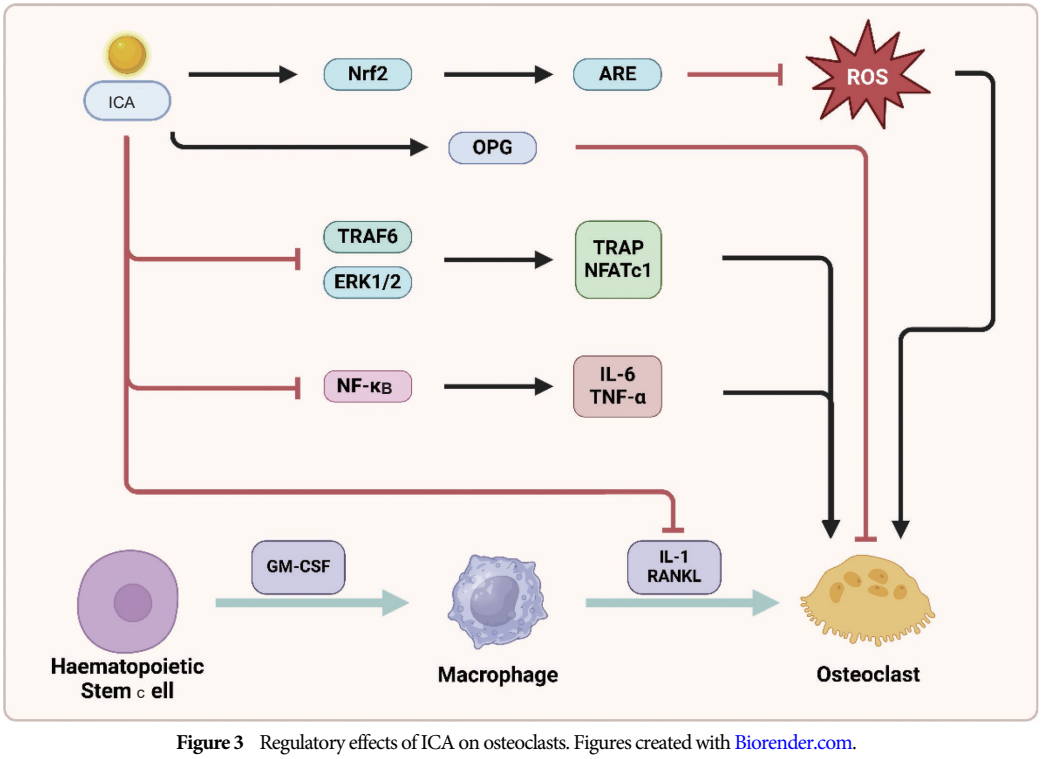

Figure 3 Regulatory effects of ICA on osteoclasts. Figures created with Biorender.com.

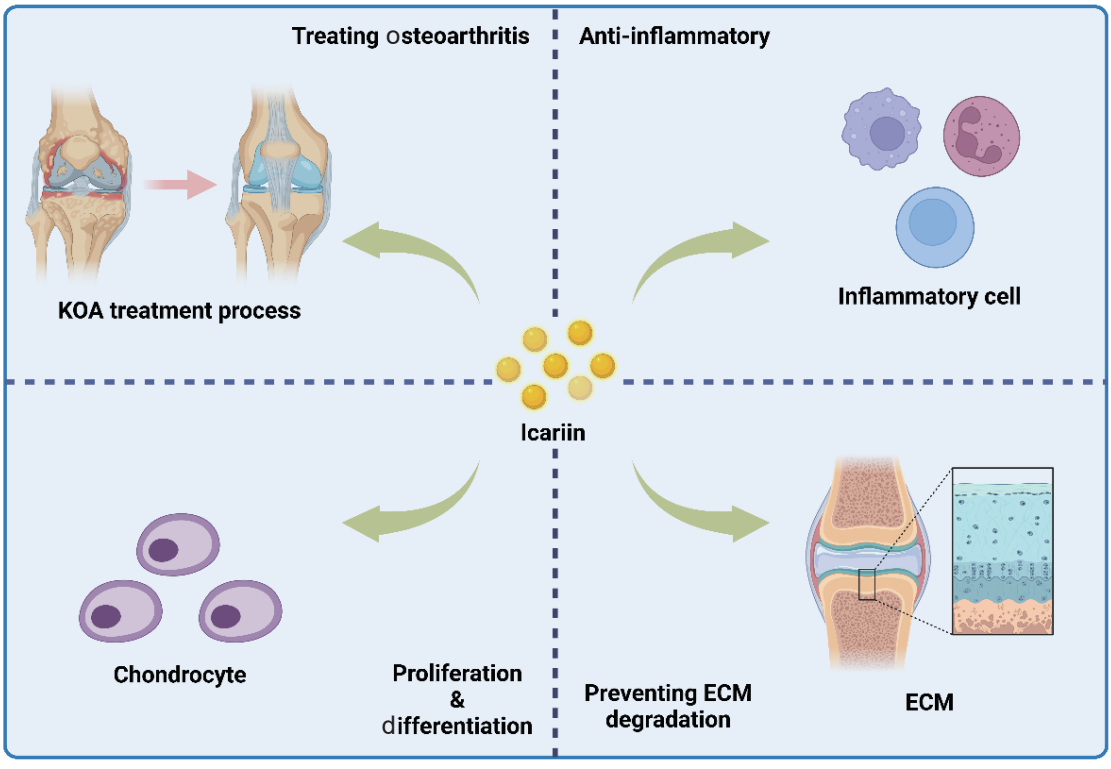

Figure 4 ICA promotes the proliferation and differentiation of chondrocytes, enhances inflammatory effects, prevents cartilage degradation, and promotes cartilage repair. Figures created with Biorender.com.

(*COX*-2), *IL-6*, and *TNF-α*, up-regulated the mRNA expression of such anti-inflammatory factors as *IL-4* and *IL-10*, and promoted the M2 polarization of macrophages to inhibit OA-related inflammation and delayed the progression of OA. Overall, ICA plays an important role in resisting inflammation, inhibiting the expression of such key inflammatory factors as IL-1β, IL-6, and TNF-α, transforming M1-type macrophages into M2-type, and promoting tissue repair.

3.2 The regulation of chondrocytes by ICA

The normal activity and proliferation of chondrocytes are important for maintaining cartilage health. It has been suggested that ICA can promote chondrocyte proliferation, inhibit chondrocyte apoptosis, and effectively alleviate cartilage degeneration in OA[76,77]. ICA accelerates cartilage regeneration by promoting the chondrogenic differentiation of BMSCs[78]. The role of ICA in regulating chondrocyte function has been increasingly studied in recent years. Sirtuin 1 (SIRT1) plays an important role in preventing the apoptosis of chondrocytes. Liu *et al.*[79] found that ICA could significantly activate the SIRT1-Nrf2-HO-1 signaling pathway in the cartilage of mice with knee OA, increase the proliferation of chondrocytes, and reduce DNA damage and apoptosis. Meanwhile, hydrogels containing ICA can also regulate the cartilage differentiation of BMSCs and promote cartilage repair in OA[80]. In addition to the apoptosis of chondrocytes, their pyroptosis and ferroptosis have also been validated to play an important role in the progression of OA. Pyroptosis, also known as cellular inflammatory necrosis, is a programmed cell death type that is dependent on inflammatory cysteoaspartic enzymes (mainly caspase-1, 4, 5, and 11), which is accompanied by the release of numerous pro-inflammatory factors. The NOD-like receptor protein 3 (NLRP3) is a key component of inflammatory vesicles. When NLRP3 is activated, it recruits and activates pro-caspase-1 to form an activated caspase-1, which cleaves gasdermin family proteins (e.g., gasdermin D (GSDMD)). This further leads to the formation of a pore in the cell membrane by the N-terminal fragment of GSDMD, which releases IL-1β and IL-18, thereby triggering pyroptosis[81]. As revealed in previous studies, the inflammatory vesicle NLRP3 plays an important role in the pathogenesis of OA. Besides, ICA can alleviate cellular pyroptosis by inhibiting NLRP3/caspase-1 signaling, and it can also increase the protein level of collagen II (Col II), thereby attenuating chondrocyte death and inhibiting the progression of OA in rats[82]. Ferroptosis is characterized by an abnormal accumulation of intracellular iron ions and ROS, which induces lipid peroxidation on the cell membrane, leading to cell death and thus playing an important role in the development of OA[83]. The down-regulated expression of GPX4 leads to an increase in the ROS level, thus triggering ferroptosis. Xiao *et al.*[84] demonstrated that ICA treatment delayed the progression of OA in rats by augmenting the SLC7A11/GPX4 signaling pathway to mitigate the ferroptosis of chondrocytes, increasing Col II synthesis in the cartilage, and attenuating ECM degradation. In addition, autophagy eliminates cellular waste and damaged components and accelerates cellular metabolism and organelle renewal[85], thus playing an important role in the regulation of chondrocytes. Besides, autophagy is essential for normal cellular metabolism and renewal of certain organelles through the lysosomal degradation process of relevant cytoplasmic proteins and damaged organelles. The autophagy level in OA chondrocytes is significantly reduced, and ICA can regulate autophagy in chondrocytes to alleviate OA by activating PI3K/AKT/mTOR signaling[86].

3.3 The regulation of the ECM of OA by ICA

The ECM of articular cartilage is mainly composed of proteoglycans, collagen, and water, and a dynamic balance

between its synthesis and decomposition can be maintained under normal physiological conditions. The increased activity and secretion of matrix metalloproteinases (MMPs) during OA disrupt the metabolic balance of the ECM and triggers the degradation of ECM proteins[87]. The degradation of the cartilage ECM is an important link in the progression of OA. Therefore, providing appropriate interventions to reduce the degradation of ECM at an early stage of OA can delay the destruction of articular cartilage, which is also one of the strategies for the early prevention and treatment of OA. Wang *et al.*[88] found that 1×10^{-6} mol/L ICA was the optimal concentration to promote chondrocyte proliferation. Under this concentration, ICA could up-regulate the expression of the SRY-box transcription factor 9 (SOX9) protein (a key factor in ECM synthesis) by modulating HIF-1α, thus increasing ECM synthesis. The severity of OA is related to the accumulation of matrix metalloproteinase 3 (MMP3) and the reduction of Col II. ICA can increase the proliferation of chondrocytes in OA, promote the protein synthesis of Col II, and inhibit the overproduction of MMP-3[89]. In human-derived chondrocytes, ICA has also been reported to delay ECM degradation by regulating the Nrf2/ARE pathway[90]. Matrix metalloproteinase 13 (MMP-13) plays an important role in ECM degradation in articular cartilage, and ICA inhibits the degradation of ECM by suppressing the expression of MMP-1, MMP-3, and MMP-13 through the MAPK pathway in an OA model[91]. ICA treatment in OA mice for 12 weeks up-regulated the expression of the collagen type II alpha 1 chain (COL2A1), down-regulated the expression of MMP-13, reduced the hypertrophy of chondrocyte, and increased the thickness of cartilage[92]. Another study also showed that ICA could inhibit the gene expression of MMP-13 and delay the degradation of articular cartilage[93]. Li *et al.*[94] developed a hydrogel containing ICA, which was implanted into the cartilage defects in rabbits, and the experimental results demonstrated that ICA up-regulated the expression of aggrecan, SOX9, and COL-II and accelerated the formation of cartilage tissues, confirming the role of ICA as an important component in cartilage tissue engineering. To improve the *in vitro* utilization of ICA and solve the low solubility and permeability of ICA. Zeng *et al.*[95] developed a BMSC-Exo carrier with higher stability and affinity, which was loaded with ICA and then injected into a rat OA model. It was found that this delivery method promoted cell proliferation and migration, inhibited the secretion of MMP13, inhibited the degradation of ECM, alleviated cartilage degradation, and accelerated cartilage tissue repair. The ECM of articular cartilage is a complex three-dimensional network structure, which provides a structural basis for the growth and metabolism of chondrocytes, thus playing an important role in the maintenance of articular cartilage health.

In summary, the inflammatory response is an important pathophysiological process in OA. ICA can inhibit the inflammatory response and protect the integrity of articular cartilage, synovium, and bone tissues, thus maintaining the normal structure and function of the joints and alleviating OA. The apoptosis, pyroptosis, and ferroptosis of chondrocytes play an important role in OA. ICA activates the autophagy of chondrocytes and regulates the function and survival of chondrocytes. Meanwhile, ICA can effectively delay the degradation of ECM in OA, which provides a theoretical basis for the clinical treatment of OA with ICA. Through the local drug delivery of slow-release ICA in patients with OA, ICA can act on deep cartilage tissues, exhibiting its potential in the clinical treatment of OA (Fig. 5).

4 The role of ICA in RA

Rheumatoid arthritis is a systemic autoimmune condition associated with a chronic inflammatory process involving the chronic inflammation of the joints and synovium. This disease is characterized by immunoregulatory destruction and leukocyte infiltration in synovial tissues, which ultimately leads to severe damage and destruction of articular cartilage and bones, thus imposing an enormous burden on society and individuals[96]. With the development of modern medicine, progress has been made in the pathological diagnosis and treatment of diseases. In RA, it is

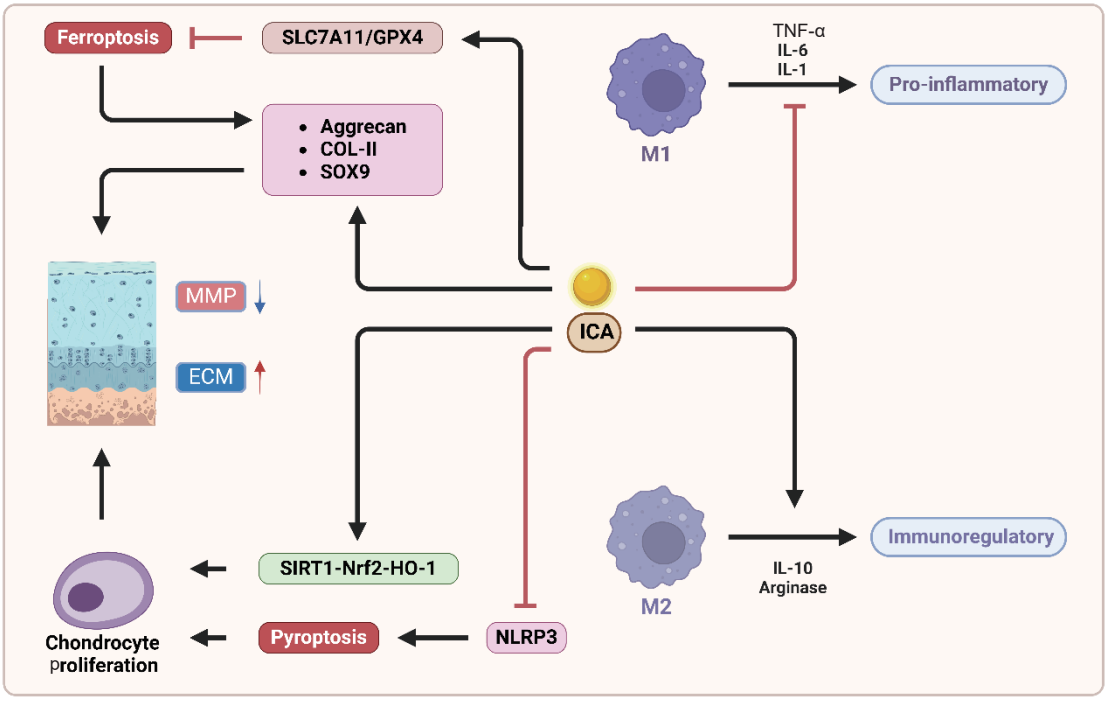

Figure 5 Mechanisms of ICA regulation of OA inflammation, chondrocytes and extracellular matrix. Figures created with Biorender.com.

only possible to decelerate the progression of the disease and improve the quality of life of patients, whereas a complete cure is not yet possible[97]. In particular, patients with RA have elevated expression levels of pro-inflammatory factors (such as IL-1β, IL-6, IL-8, TNF-α, and IL-17) in the blood and synovial fluid[98-100]. In addition, at the inflammatory response site, inflammatory cell infiltration promotes the proliferation of fibroblast-like synoviocytes (FLSs) in the joint, leading to the development and exacerbation of chronic inflammation.

As previously mentioned, ICA plays a role in anti-inflammation and modulation of immune responses. ICA has been shown to inhibit the proliferation of fibroblast-like synoviocytes (RA-FLSs) and downregulate the secretion of TNF-α, IL-1β, and IL-6, likely by promoting apoptosis and exerting anti-inflammatory effects *via* the miR-223-3p/NLRP3 signaling pathway[101]. The results of experiments on the extraction of the synovium of RA mice also indicated that ICA inhibited the activity of FLSs in a dose-dependent manner, while the expression of NLRP3 and Bcl-2 was down-regulated, and the arthropathy in these mice became less severe[102]. For the regulation of synoviocytes in RA, another experiment provided a different insight, namely that ICA could inhibit the ferroptosis of synoviocytes and increase cell viability by activating the Xc-/GPX4 axis[103]. Elevated IL-17 is associated with RA. Chi *et al.*[104] corroborated that ICA reduced the production of Th17 cells and inhibited the mRNA expression of IL-17, while the serum IgG2a level in ICA-treated mice decreased significantly, which delayed the progression of RA in mice. This result suggested that ICA exerted anti-inflammatory effects in the immunomodulation process. In addition, Pu *et al.*[105] also demonstrated that ICA could inhibit the proliferation and migration of FLSs and increase the apoptosis of FLSs by decreasing the mitochondrial membrane potential and up-regulating the levels of cytoplasmic cytochrome c and intracellular ROS through ICA. Wu *et al.*[106] collected synovial tissues of active RA, and the experimental results revealed that ICA inhibited the NF-κB signaling pathway by up-regulating the expression of TRIB1, and it also attenuated the inflammatory response of RA-FLSs. The destruction of articular cartilage by RA causes knee joint pain and dysfunction, and it is a common reason for joint replacement at the advanced stage of RA in clinical practice. After the treatment with ICA, the number of vesicles in chondrocytes decreased significantly, the rough endoplasmic reticulum was abundant, and the filamentous pseudopods on the surface were larger and wider. Besides, ICA could protect chondrocytes from damage and reduce cartilage destruction in RA[107]. Tissue proteinase K is expressed by OCs and FLSs, and the serum concentration of proteinase K is higher in patients with RA than in the healthy population. The overexpression of tissue proteinase K in mice can lead to synovitis and cartilage and bone destruction. Sun *et al.*[108] conducted an *in vitro* model experiment and found that ICA inhibited the activity of tissue proteinase K in a dose-dependent manner, decreased the concentration of CTX-I, reduced joint cartilage degradation, and delayed the progression of RA. The increased number of M1 macrophages in synoviocytes in the early stage of RA is one of the causes of synovial inflammation in RA. Yan *et al.*[109] developed an adipose stem cell-exosome (ADSCs-EXO) carrying ICA for the treatment of RA. The experimental results showed that ADSCs-EXO-ICA significantly reduced the protein concentrations of TNF-α, IL-1β, and IL-6, inhibited the proliferation of M1 macrophages, and promoted the phenotypic transition from M1 to M2 by inhibiting the ERK/HIF-1α/GLUT1 pathway. ADSCs-EXO-ICA also down-regulated the expression of rheumatoid factors, which further alleviated RA.

The above studies indicate that ICA can play a role in the treatment of synovial inflammation and cartilage degradation in RA by decreasing the proliferative capacity of RA-FLSs, inhibiting the expression of such factors as TNF-α, IL-1β, and IL-6, and regulating the polarization status of macrophages, as well as regulating the immune response by down-regulating the serum IgG2a expression (Fig. 6). Nevertheless, the regulation of ICA for

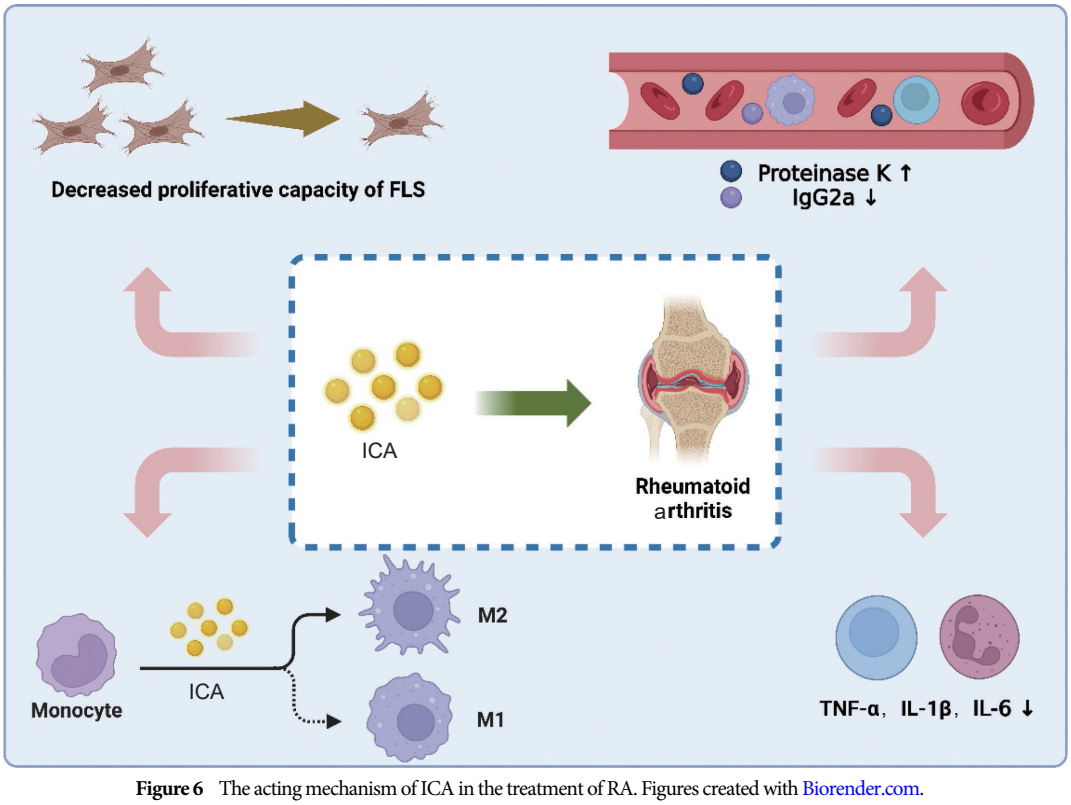

Figure 6 The acting mechanism of ICA in the treatment of RA. Figures created with Biorender.com.

the immune response in RA has not been sufficiently clarified, and an in-depth exploration of relevant acting mechanisms is required to better utilize the medicinal value of ICA in the treatment of RA.

5 The role of ICA in IVDD

The intervertebral disc is a nonvascular structure composed of fibrous tissues and cartilage, which can connect two adjacent vertebrae, cushion the spine from mechanical stress, and allow the flexion, extension, bending, and rotation of the spine[110]. The degeneration of intervertebral discs is associated with advanced age, obesity, and overuse[4]. Low back pain is a common clinical manifestation of degenerative disc pathology, affecting approximately 700 million individuals worldwide and inducing more than 30% of events related to absenteeism. The degenerative disc disease is mainly manifested as decreased disc height, fibrous annulus tears, proteoglycan loss, nucleus pulposus (NP) dehydration, and calcification of cartilage endplates[111]. Currently, IVDD is mainly treated to control symptoms and reduce disability, emphasizing the importance of early treatment[112]. Therefore, a prompt pharmacologic intervention to prevent the apoptosis of medullary cells and their ECM degradation is an effective therapeutic strategy.

ICA was continuously applied to rabbit annulus fibrosus cells, cell proliferation, and proteoglycan expression, and the proportion of S-phase cells in rabbit annulus fibrosus cells increased significantly compared with the blank group[113]. Sun *et al.*[114] conducted a network pharmacological analysis of ICA and IVDD. They found that ICA may be used to treat intervertebral disc degenerative diseases by modulating inflammatory and immune responses. IL-1β is one of the inflammatory mediators that can stimulate NP cell apoptosis and ECM degradation. ICA can inhibit the IL-1β-induced activation of the MAPK and NF-κB-related signaling pathways and suppress the IL-1β-induced production of degradative enzymes, thus playing a role in protecting NP cells and ECM[115]. ROS is mainly produced in mitochondria, and the disruption of intracellular redox homeostasis by excessive ROS may lead to cellular inflammation, apoptosis, autophagy, and senescence[116], thereby causing ECM decomposition and IVDD. For this reason, Hua *et al.*[117] applied ICA to H_2O_2-stimulated human myeloid cells. They found that ICA reduced H_2O_2-induced apoptosis of myeloid cells, inhibited H_2O_2-induced ROS production, activated Nrf-2 protein in human NP cells, and significantly decelerated the progression of IVDD in a rat model. Deng *et al.*[118] also found similar results that ICA could reduce IL-1β-induced apoptosis of human myeloid cells by regulating the PI3K/AKT pathway, decrease the abnormal accumulation of ROS in human myeloid cells, restore the mitochondrial membrane potential, and mitigate mitochondrial damage. The cartilage endplate (CEP) consists of a layer of cartilage tissues, and apoptosis and ECM degradation of the CEP are the direct causes of CEP degeneration. Shao *et al.*[119] showed that ICA could alleviate OS and mitochondrial dysfunction in CEP chondrocytes, improve cell survival, and relieve cartilage degeneration and calcification by regulating the Nrf-2/HO-1-mediated activation of mitochondrial autophagy and ferroptosis, thus improving IVDD. He *et al.*[120] treated a rabbit model related to intervertebral disc degeneration with ICA (3 mg/(kg·day)) by gavage for 4 weeks. The results indicated that the protein expression of TIMP-1 and Bcl-2 was up-regulated, while that of MMP-13 and Bax was down-regulated in the intervertebral discs, which prevented and controlled the degeneration of intervertebral discs.

In summary, ICA can delay the progression of IVDD by alleviating inflammation, reducing ECM degradation, improving mitochondrial function, and scavenging ROS. Meanwhile, ICA significantly promotes the proliferation of NP cells, reduces senescence and apoptosis, and protects the ECM of the intervertebral disc (Fig. 7). Currently, there are fewer experimental studies on the application of ICA to the treatment of IVDD. Based on the results of the above studies, it can be expected that ICA will be an effective alternative drug for the treatment of IVDD.

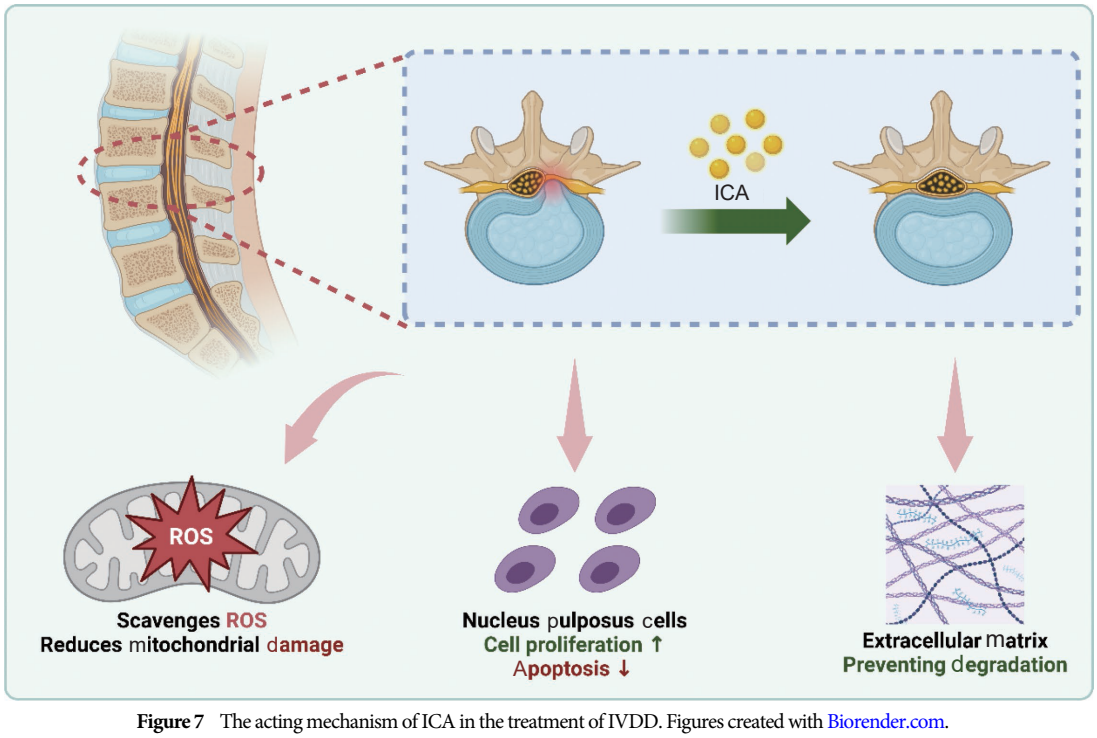

Figure 7 The acting mechanism of ICA in the treatment of IVDD. Figures created with Biorender.com.

6 Conclusion and outlook

The long-term administration of drugs for the treatment of chronic bone and joint diseases will inevitably bring new side effects, and new therapeutic drugs are being developed. According to TCM, ICA, a main ingredient extracted from *Epimedium* based on modern technology, can tonify the kidney, enhance Yang, strengthen muscles and bones, dispel wind-dampness, and exert other therapeutic effects. It has been demonstrated that ICA can promote the proliferation and differentiation of OBs, inhibit the activity of OCs, and inhibit bone resorption while promoting bone formation, thus playing a role in the treatment of OP. Meanwhile, ICA can regulate the function of chondrocytes, promote the regeneration of cartilage, inhibit the ECM degradation of cartilage, exert immunoregulatory effects, down-regulate the expression of inflammatory factors, and eliminate ROS. Besides, it can also inhibit the destruction of articular cartilage resulting from inflammation, improve the microenvironment of inflammation, and play a role in the treatment of OA and RA. In addition, ICA can also act on the NP cells by increasing their activity and inhibiting apoptosis, thus decelerating the progression of degenerative IVDD. Therefore, ICA has great medicinal value in the field of orthopedics.

In recent years, ICA has become a popular research topic as a potential therapeutic agent for chronic bone and joint diseases. However, there are some shortcomings in the experimental study of ICA. Firstly, the growth of *Epimedium* is affected by such objective factors as planting location, temperature, and humidity, which may change the content of ICA in the herb. Secondly, there is a lack of specific targets to regulate chronic bone and joint diseases from the acting mechanism of ICA. The focus of existing studies on ICA is placed on the cellular level and animal experiments, and there is a lack of long-term clinical cohort studies and retrospective analyses to assess its efficacy and safety. Moreover, the large-scale clinical application of ICA is also limited by its low solubility, poor bioavailability, and low permeability. Optimizing the route, dosage, and frequency of administration of *Epimedium* for human use to achieve the best clinical therapeutic effect has not been adequately addressed. With the development of modern biomedical technology, further explorations may be conducted from the research and development of highly effective, long-lasting, and therapeutically accurate ICA biologics. These efforts may contribute to the extensive application of ICA in clinical practice and benefit more patients with chronic bone and joint diseases through TCM-based treatment.

Data availability

Additional data related to this paper may be requested from the corresponding author upon request.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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Author contribution statement

YZ, FY, HQ: Data curation, project administration, validation, writing manuscript. JC, QY, JH, GZ, TQ, SY, YZ, JW: Data curation, project administration, validation. FY, HZ: funding acquisition. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

None.

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