

# Programmed Cell Death of Chondrocytes, Synovial Cells, Osteoclasts, and Subchondral Bone Cells in Osteoarthritis

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**Abstract:** Osteoarthritis (OA) is a common and debilitating chronic disease characterized by severe inflammation and progressive damage to adjacent tissues and cartilage. Traditional risk factors such as obesity, gender, and aging have long been recognized as contributing factors to osteoarthritis. Emerging evidence highlights that the dysregulation of programmed cell death (PCD) plays a crucial role in the pathogenesis and progression of this disease. Numerous studies have shown that various forms of programmed cell death, including ferroptosis, pyroptosis, autophagy, cuproptosis, and apoptosis, are closely associated with osteoarthritis. Ferroptosis is an iron-dependent cell death driven by lipid peroxidation, which is related to iron overload and oxidative stress in osteoarthritis, leading to chondrocyte dysfunction and cartilage degradation. Pyroptosis, an inflammatory cell death, is triggered by the activation of inflammasomes, promoting the release of pro-inflammatory cytokines, exacerbating joint inflammation, and accelerating disease progression. Autophagy, a cellular self-degradation process, has a dual role in osteoarthritis: it acts as a protective mechanism against stress in the early stage, but when autophagy is dysregulated, it promotes cartilage degeneration. Cuproptosis is a newly discovered copper-dependent cell death pathway, and since copper metabolism dysregulation affects the function of bone and cartilage cells, it is associated with osteoarthritis. Apoptosis is an actively regulated cell death process controlled by genes and is mediated by two main pathways. The extrinsic pathway is activated when death ligands bind to receptors, triggering the activation of caspase-8 and caspase-3; the intrinsic pathway is initiated by cellular stress factors such as DNA damage, leading to mitochondrial damage and the activation of caspase-9 and caspase-3. In osteoarthritis, inflammatory factors and oxidative stress activate these two pathways, accelerating the apoptosis of chondrocytes and disease progression. This review systematically elaborates on these different types of programmed cell death and their specific roles in the development and progression of osteoarthritis. It also delves into the latest research on the molecular mechanisms of these programmed cell death pathways in the context of osteoarthritis, clarifying how they interact with other cellular processes to drive disease development. In addition, the review summarizes the clinical applications of therapeutic methods targeting programmed cell death in osteoarthritis. Ingredients from traditional Chinese medicine and other drugs show potential in regulating ferroptosis, pyroptosis, autophagy, cuproptosis, and apoptosis to alleviate the symptoms of osteoarthritis. For example, *Icariin* and *Myristicin* can prevent ferroptosis, while *Matrine* and metformin can reduce pyroptosis. Regarding cuproptosis, copper chelators and copper ion carriers are also under investigation. Therapeutic strategies targeting mitochondrial autophagy and copper balance also offer hope for the treatment of osteoarthritis. Currently, non-coding RNAs, phytochemicals, and some proteins have been explored for their ability to inhibit the apoptosis of chondrocytes. In conclusion, a deep understanding of the mechanisms of programmed cell death in osteoarthritis not only provides new perspectives on the pathogenesis of the disease but also points the way for the development of targeted treatment strategies and the improvement of the treatment outcomes for osteoarthritis.

**Keywords:** osteoarthritis, ferroptosis, pyroptosis, autophagy, cuproptosis, apoptosis

## Introduction

OA is one of the most common chronic joint diseases and a major cause of disability in the elderly population worldwide. According to statistics, more than 500 million people worldwide are affected by OA.<sup>1</sup> Its prevalence increases

significantly with age, and it has become one of the major public health issues affecting the quality of life and imposing a health - related economic burden on people. OA was previously considered a simple degenerative disease of cartilage in its early stage.<sup>2</sup> However, research in recent years has shown that OA is actually a whole - joint disorder. Its pathological process not only involves the destruction of articular cartilage and the remodeling of subchondral bone structure, but also includes meniscus degeneration, synovial inflammation, and changes in the structure and function of the infrapatellar fat pad.<sup>3-5</sup> The clinical manifestations of OA mainly include joint pain, stiffness, swelling, and limited mobility, which severely affect the daily lives of patients. The pathogenesis of OA is complex and is affected by the combined action of multiple factors, including abnormal mechanical loading, genetic background, metabolic disorders, and chronic inflammatory responses.<sup>6</sup> Common risk factors include aging, obesity, joint trauma, changes in female hormone levels, joint instability, and metabolic syndrome.<sup>7,8</sup> During the progression of OA, chronic low - grade inflammation in the joint microenvironment plays an important role.<sup>9</sup> Pro - inflammatory factors such as IL - 1 $\beta$ , TNF -  $\alpha$ , and IL - 6 can induce enhanced catabolism in chondrocytes while inhibiting anabolism, thus disrupting joint homeostasis.<sup>10</sup> In addition, the increased expression of matrix metalloproteinases (such as MMP13) and aggrecanases accelerates the degradation of the extracellular matrix (ECM), leading to irreversible damage to cartilage.<sup>11</sup> Currently, there is no definite cure for OA. Clinical treatment mainly focuses on relieving symptoms, and commonly used medications include non - steroidal anti - inflammatory drugs (NSAIDs), analgesics, and intra - articular injection of glucocorticoids.<sup>12</sup> However, these treatment methods cannot reverse the course of the disease, and long-term use may also lead to side effects such as hepatotoxicity, nephrotoxicity, and adverse cardiovascular reactions.<sup>13</sup> Therefore, there is an urgent need to deeply explore the molecular mechanisms of OA in order to identify new therapeutic targets and intervention strategies.

In recent years, programmed cell death (PCD), as an important mechanism for regulating cell fate, has gradually become a new research hotspot in the field of OA. PCD includes various forms, such as pyroptosis, ferroptosis, autophagy, the newly proposed cuproptosis, and apoptosis. These forms of cell death play a crucial role in maintaining the homeostasis and pathological changes of the joint tissues in OA.<sup>14</sup> Studies have shown that the expression of NLRP3 inflammasome related to pyroptosis in the synovial fluid of patients with OA is significantly upregulated, which promotes the release of IL-1 $\beta$  and IL-18, thus exacerbating synovial inflammation and cartilage destruction.<sup>15</sup> On the other hand, the P2X7 receptor can promote the assembly of the NLRP3 inflammasome, further inducing the process of pyroptosis.<sup>16</sup> Ferroptosis is a process of cell death caused by lipid peroxidation damage driven by iron ions. The decrease in the expression of GPX4 in OA chondrocytes will increase their sensitivity to ferroptosis, leading to oxidative stress and matrix degradation.<sup>17</sup> In addition, ferroptosis is closely related to the upregulation of MMP13 and the downregulation of type II collagen expression, leading to further degeneration of cartilage.<sup>18</sup> In contrast, autophagy, as a protective mechanism for maintaining cellular homeostasis and responding to stress, a decrease in its activity is considered an important contributing factor to the progression of OA.<sup>19</sup> Cuproptosis is a newly discovered type of cell death in recent years, and its mechanism is related to the protein stress caused by the excessive accumulation of copper ions in the mitochondria of cells. Although there is relatively little research on its role in OA, preliminary evidence shows that cell types related to immune regulation (such as dendritic cells, mast cells, and CCR2-positive monocytes) may participate in the immune response process of OA by regulating cuproptosis-related genes.<sup>20-23</sup> Apoptosis also plays a crucial role in osteoarthritis. Exogenous inflammatory factors can activate the death receptor pathway, leading to apoptosis of chondrocytes.<sup>24</sup> Endogenous factors such as DNA damage and oxidative stress trigger the mitochondrial apoptosis pathway, causing the release of cytochrome C and activating the caspase cascade, which accelerates the apoptosis of chondrocytes and promotes the progression of osteoarthritis.<sup>25</sup> In conclusion, various forms of programmed cell death play an important role in the onset and progression of OA. They may not only mediate tissue damage but also serve as potential protective mechanisms. A thorough exploration of their molecular mechanisms and action pathways is expected to provide new strategies for the early intervention and targeted treatment of OA. This review aims to systematically sort out the latest research progress of pyroptosis, ferroptosis, autophagy, cuproptosis, and apoptosis in OA, and analyze their potential molecular targets and therapeutic values.

## Research Method

This review systematically searched databases such as PubMed and Web of Science, using keywords including “Osteoarthritis”, “Programmed Cell Death”, “Pyroptosis”, “Ferroptosis”, “Autophagy”, and “Cuproptosis”. Relevant

literatures published before April 2025 were screened. The research related to the pathogenesis of OA, the regulatory pathways of cell death, and potential therapeutic targets was mainly collected. Peer-reviewed research papers and the latest review articles were preferentially included.

## The Mechanism of Osteoarthritis

The dysregulation of cytokines is a key factor in the progression of OA. Cytokines that promote inflammation can persistently damage chondrocytes and disrupt the metabolic balance of the extracellular matrix in cartilage.<sup>26</sup> This mechanism triggers catabolic enzymes such as matrix metalloproteinases (MMPs) and aggrecanases (ADAMTS), ultimately leading to the degradation of cartilage and other joint structures.<sup>27</sup> The primary inflammatory mediators that play a role in the onset of OA include IL-1 $\beta$ , TNF- $\alpha$ , and IL-6.<sup>28</sup> They act as activators for different signaling pathways, which enables them to stimulate other cytokines and contribute to the advancement of the pathological processes linked to OA.<sup>29</sup> During this process, chemokines play an important role as well. When stimulated by cytokines, they draw inflammatory cells to the joint, leading to an increased presence of these cells in the joint area.<sup>30</sup> To sum up, chemokines can facilitate the release of inflammatory substances and contribute to the progression of OA.<sup>30</sup> IL-1 $\beta$  is an important pro-inflammatory cytokine that significantly contributes to the progression of several diseases. It first binds to its receptor (IL-1RI), which activates signaling pathways like NF- $\kappa$ B and MAPK.<sup>31</sup> This activation causes an increase in ADAMTS and MMPs, resulting in enhanced breakdown of proteoglycans and more extensive collagen damage in the joint, ultimately leading to increased joint injury.<sup>32</sup> Moreover, IL-1 $\beta$  influences the same signaling pathway by lowering SOX-9 levels, which in turn decreases the production of type II collagen. It also enhances the production of COX-2, PGE-2, and NO, leading to a decline in proteoglycan synthesis. IL-1 $\beta$  is recognized for increasing the levels of several chemokines, such as IL-8, CCL2, and CCL5, in addition to cytokines like IL-6 and TNF- $\alpha$ .<sup>33</sup> This mechanism establishes a positive feedback loop that intensifies the inflammatory response and leads to a higher production and release of IL-1 $\beta$ .<sup>27</sup> In conjunction with IL-1 $\beta$ , TNF- $\alpha$  is also acknowledged as a key pro-inflammatory cytokine that plays a role in the progression of OA.<sup>34,35</sup> The degeneration of cartilage and various joint components can be induced by these two cytokines, which in turn facilitates the progression of OA. TNF- $\alpha$  can bind to TNFRF-1, resulting in the creation of two separate signaling complexes.<sup>36</sup> Complex 1 supports cell survival and increases the levels of NF- $\kappa$ B, MAPK, and AP-1. This leads to the degradation of proteoglycans and subsequent harm to collagen, while also inhibiting the synthesis of both proteoglycans and collagen.<sup>37</sup> The activation of complex 2 can initiate a series of reactions that produce FADD, which then activates procaspase 8/10 and caspase 3, ultimately resulting in cell apoptosis.<sup>38</sup> Additionally, TNF- $\alpha$  engages with another receptor called TNRF-2, activating the inflammation-related signaling pathway JNK and resulting in the release of NF- $\kappa$ B. IL-6, a significant pro-inflammatory cytokine, operates via the mediator gp130 by attaching to either IL-6R or sIL-6R to produce its biological effects.<sup>39</sup> As a result, Gp130 activates intracellular signaling pathways that regulate inflammatory responses, the production of enzymes, and the expression of collagen and proteoglycans. IL-6 activates the PI3K, JAK/STAT, and MAPK signaling pathways via traditional signal transduction and trans-signaling methods. This activation influences the production of enzymes like TIMP, MMPs, and ADAMTS, as well as type II collagen and proteoglycans.<sup>40</sup>

Chemokines are tiny signaling molecules that can initiate chemotaxis, helping immune effector cells move and orient themselves towards sites of infection or inflammation. Consequently, chemokines play a vital role in maintaining joint inflammation linked to OA.<sup>30</sup> Chemokines from the CC family, including CCL2, CCL3, CCL4, and CCL5, are important contributors to the development of OA.<sup>30</sup> Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a strong attractant for monocytes, aiding in the recruitment of memory T cells and natural killer (NK) cells. Its main function is linked to its engagement with the CCR2 receptor.<sup>41</sup> CCL2 increases the levels of MMP-3, leading to a reduction in proteoglycans and the degradation of cartilage tissue.<sup>42</sup> CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ), and CCL5 (RANTES) are other members of the CC chemokine family that exhibit heightened expression in OA. Studies show that people with radiographic knee OA before surgery have significantly different levels of CCL3 in their blood compared to a control group.<sup>43</sup> As the disease progresses, the levels of CCL3 in plasma increase. Similar studies have shown that CCL5 levels in the synovial fluid of OA patients are considerably elevated compared to the control group.<sup>44</sup> It is noteworthy that all three chemokines act as ligands for CCR5. Additionally, the CXC family of chemokines, particularly CXCL8 (also referred to as IL-8) and

CXCL12, play important roles in the progression of OA.<sup>45</sup> CXCL8 engages with the CXCR1 and CXCR2 receptors present on white blood cells and also affects chondrocytes, osteoclasts, fibroblasts, epithelial cells, and several other cell types.<sup>45–47</sup> Studies using the human chondrocyte cell line (CHON-002) have shown that TNF- $\alpha$  can enhance the expression of CXCL8.<sup>48</sup> Furthermore, advanced glycation end products (AGEs) are capable of activating the signaling route of NF- $\kappa$ B, leading to an increased production of CXCL8. This chemokine tends to build up in cartilage as people age and encourages the enhancement of catabolic activities in chondrocytes.<sup>49</sup> Furthermore, studies show that in chondrocytes from individuals with OA, IL-8 can increase the levels of collagen I, MMP1, and MMP-13 proteins, and it also facilitates the phosphorylation of STAT3 and the p65 subunit of NF- $\kappa$ B.<sup>48</sup> It affects the shape of chondrocytes by lowering internal GTP-Cdc42 levels and increasing the formation of stress fibers. IL-8 promotes the growth of chondrocytes and aids in the mineralization process of the extracellular matrix.<sup>46</sup> CXCL12, also known as stromal cell-derived factor-1 (SDF-1), is a chemokine crucial for tissue repair. It binds to CXCR4 and draws mesenchymal stem cells (MSCs) to sites of injury.<sup>50</sup> CCL21 is also an important inflammatory mediator, which is secreted by cartilage, meniscus, and synovial membrane. In the joint microenvironment, CCL21 contributes to the recruitment of immune cells and the regulation of local inflammatory responses. Its role in the diseased joint tissues may significantly affect the progression and severity of inflammation, and it has a crucial impact on the overall health of the joints.<sup>51</sup> The inflammation of the infrapatellar fat pad and synovium, as important aspects in the pathogenesis of OA, has a complex relationship with the dysregulation of cytokines and chemokines. The inflammation of the infrapatellar fat pad can release a variety of cytokines and adipocytokines, further exacerbating the inflammatory microenvironment within the joint.<sup>3–5</sup> This not only aggravates the damage to chondrocytes and the extracellular matrix but also promotes the infiltration of inflammatory cells. Similarly, synovial inflammation is a key feature of OA. Synovial fibroblasts and macrophages will release a series of cytokines, chemokines, and proteases, leading to cartilage degradation and joint destruction.<sup>52</sup> Articular cartilage consists of chondrocytes along with the extracellular matrix (ECM) that surrounds them. Chondrocytes play an essential role in preserving the stability of collagen metabolism at a low turnover rate, provided there is no pathological damage.<sup>53</sup> At the same time, a growing body of research indicates that the deterioration of cartilage is a major pathological alteration in OA, likely resulting from the breakdown of the ECM due to metabolic imbalances in chondrocytes.<sup>54</sup>

The above-mentioned inflammatory processes do not occur in isolation but are closely related to the elaborated mechanisms of programmed cell death. Programmed cell death, including ferroptosis, pyroptosis, autophagy, cuproptosis, and apoptosis can be triggered by inflammatory cytokines and chemokines.<sup>55</sup> Conversely, it can also regulate the production and release of these factors. For example, in an inflamed joint, elevated levels of IL-1 $\beta$  and TNF- $\alpha$  can induce pyroptosis in chondrocytes, further disrupting the cartilage structure. On the other hand, the activation of autophagy triggered by inflammatory stress may attempt to maintain intracellular homeostasis and protect chondrocytes.<sup>14</sup>

## The Research Progress of Programmed Cell Death in Osteoarthritis

### Ferroptosis and Osteoarthritis

#### Overview of Ferroptosis

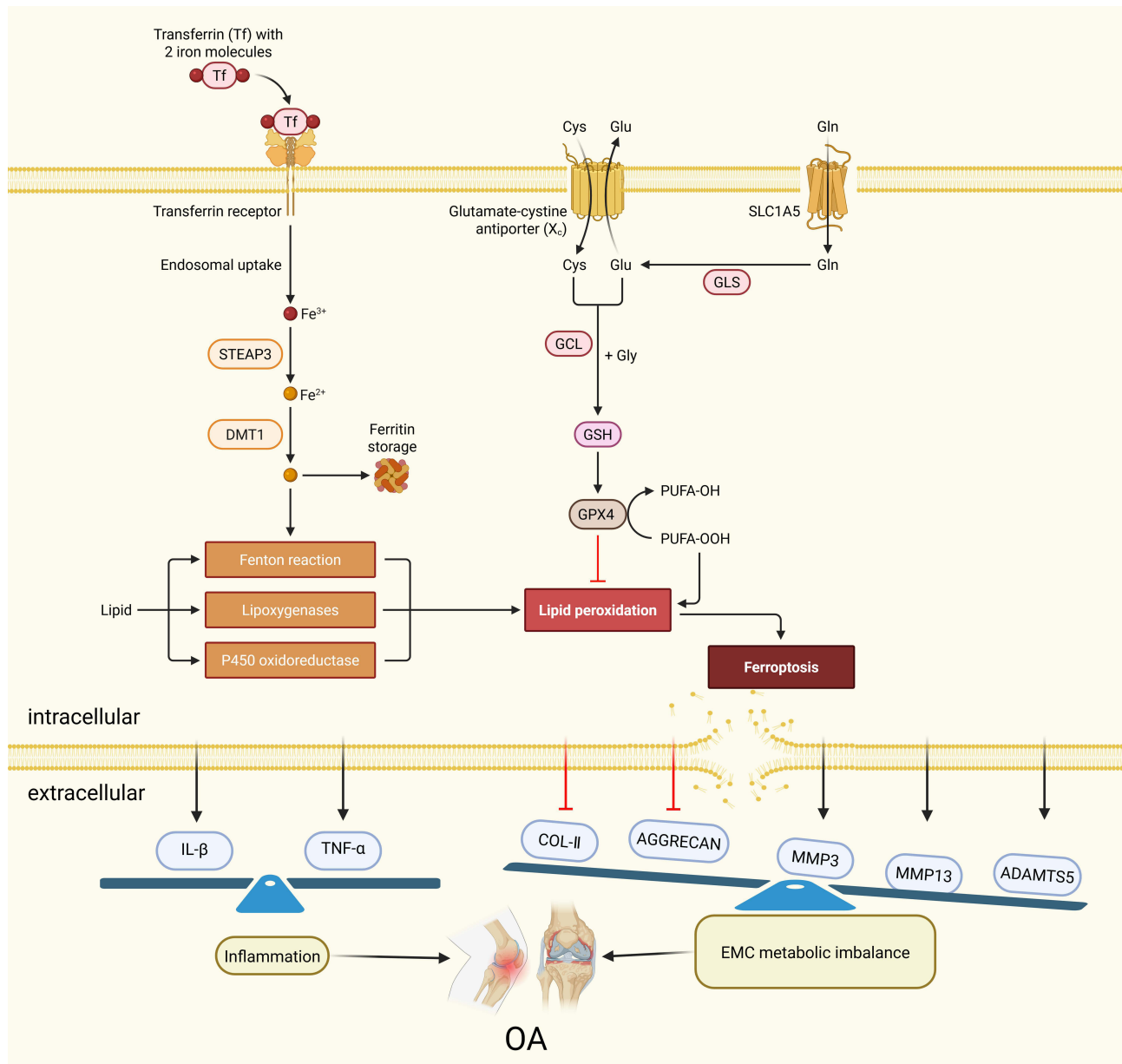
Ferroptosis is a new kind of programmed cell death which is caused by the accumulation of lipid peroxides dependent on iron. The mechanisms involved are mainly linked to disruptions in iron metabolism, the buildup of lipid peroxides, and an imbalance in the amino acid antioxidant system. Fe<sup>3+</sup> attaches to transferrin (TF) in the blood and transports the complex into the cell's nuclear endosome via transferrin receptor 1 (TfR1) located on the cell membrane.<sup>56</sup> Within the endoplasmic reticulum, trivalent iron ions (Fe<sup>3+</sup>) are separated from their complex and converted to Fe<sup>2+</sup> by the enzyme prostate stromal epithelial antigen 3 (STEAP3). These ferrous ions are subsequently transported into the cytoplasm via divalent metal transporter 1 (DMT1) or some members of the zinc-iron regulatory protein family, namely ZIP8/14.<sup>57</sup> In the cytoplasm, a small amount of Fe<sup>2+</sup> exists in the soluble iron pool (LIP), while the majority binds to the light chain of ferritin (FTL) and heavy chain 1 (FTH1) to form ferritin for storage. Ferritin (also designated as FPN or SLC40A1) has the ability to export excessive Fe<sup>2+</sup> from the cell and convert it to Fe<sup>3+</sup>, thereby facilitating the regulation of iron levels within the cytoplasm.<sup>58</sup> The accumulation of active Fe<sup>2+</sup> has the potential to initiate ferroptosis through the generation of ROS by the Fenton reaction or by directly promoting lipid oxidation processes while serving as a cofactor for

lipoxygenase (LOX).<sup>59</sup> Two important lipid peroxidation metabolites are reactive oxygen species (ROS) and lipids. ROS are mainly generated by the Fenton reaction, while the other two methods involve oxidative processes occurring in mitochondria and enzymatic actions carried out by the NADPH-dependent oxidase (NOX) family.<sup>60</sup> Arachidonic acid (AA), an important form of polyunsaturated fatty acids (PUFAs), acts as the main substrate for lipid peroxidation during ferroptosis. Polyunsaturated fatty acyls (PL-PUFAs) serve as key substrates for lipid peroxidation and play a role in the following processes of iron movement,<sup>17,61,62</sup> the process can occur through either an enzymatic method or a non-enzymatic method. In the non-enzymatic process, hydroxyl radicals ( $\text{HO}^\cdot$ ) produced by the Fenton reaction involving  $\text{Fe}^{2+}$  can remove hydrogen atoms from lipids, leading to the formation of lipid radicals. These lipid radicals then generate peroxy radicals, which play a role in non-enzymatic lipid peroxidation.<sup>63</sup> Researchers have discovered at least two separate pathways in the enzymatic process. One pathway features iron-dependent lipoxygenases (LOXs), which can transform polyunsaturated fatty acids (PUFAs) into peroxides, leading to the formation of several derivatives such as malondialdehyde (MDA) and 4-hydroxy nonadecenoic acid (4-HNE), among others.<sup>60</sup> Studies have shown that cytochrome P450 oxidoreductase (POR) plays a role in lipid peroxidation through the alternative enzyme pathway, potentially leading to ferroptosis.<sup>64</sup> Certainly, this process requires iron to act as a catalyst. Lipid peroxidation can cause enzymes at the membrane to malfunction, potentially altering the fluidity and permeability of the plasma membrane.<sup>65</sup> When lipid peroxidation occurs, cells initiate an antioxidant response to counteract its effects and maintain balance. This response mainly involves cysteine/glutamate antiporters, referred to as the Xc-system, and GPX4.<sup>66</sup> The Xc-system consists of solute carrier family 7 member 11 (SLC7A11) and solute carrier family 3 member 2 (SLC3A2). It enables the transport of glutamate (Glu) out of the cell and permits the entry of cysteine.<sup>67</sup> Cysteine is then converted into cysteine, which serves as a building block for the synthesis of glutathione (GSH). Cells absorb glutamine (Gln) through the SLC1A5 transporter protein, and it is then converted into glutamate (Glu) by the enzyme glutaminase (GLS), which affects the consumption rate of Glutathione (GSH). GSH is a tripeptide that consists of glutamate, cysteine, and glycine.<sup>68</sup> It is produced by the enzyme GCL ( $\gamma$ -glutamylcysteine synthetase) and is crucial as a cofactor for GPX4 in the process of detoxifying lipid peroxides. GPX4 is the sole enzyme capable of transforming GSH into its oxidized form, known as GSSG, while also reducing lipid peroxides to their respective alcohols.<sup>59</sup> As a result, GPX4 and SLC7A11 can be regarded as crucial regulators of ferroptosis. [Figure 1](#).

## The Role of Ferroptosis in Osteoarthritis

### Iron Overload and Osteoarthritis

The basic pathological mechanisms of OA include damage to the cartilage at the joint margins and the subchondral bone, accompanied by synovitis, in which chondrocytes play an important role. In addition, the meniscus and the infrapatellar fat pad are also of great significance.<sup>69</sup> The meniscus is a key structure for the distribution of joint loads. In OA, it undergoes degeneration, disrupting the normal joint mechanics and imposing abnormal pressure on the cartilage and subchondral bone. When the infrapatellar fat pad is inflamed, it secretes inflammatory mediators, affecting the function of chondrocytes and leading to synovitis, which further exacerbates the progression of OA.<sup>69,70</sup> Research has shown that the iron levels in the synovial fluid of individuals with OA are elevated.<sup>71</sup> However, when compared to the iron levels in the synovial fluid of patients with rheumatoid arthritis (RA), the increase in OA patients is less pronounced.<sup>71</sup> Compared to the healthy control group, patients with OA showed higher serum ferritin levels, indicating more severe joint imaging results.<sup>72</sup> Hereditary hemochromatosis is characterized by an excess buildup of iron in the body, which often results in patients suffering from OA.<sup>73</sup> Jing and their team developed a mouse model exhibiting iron overload (IO) to explore the role of iron in the progression of OA.<sup>71</sup> In the knee joint, compared to the control group, the IO model showed greater cartilage degradation, harm to the subchondral bone, and heightened sclerosis, leading to higher OARSI scores for OA. They observed an increase in the expression levels of ADAMTS5 and MMP13, as well as a rise in the number of osteoclasts. To explore the possible molecular mechanisms connecting inflammation and iron overload in OA, scientists exposed chondrocytes to IL-1 $\beta$  or TNF- $\alpha$ . They observed an increase in TfR1 and DMT1, which are transporters for metal ions, while FPN, the protein that facilitates iron export, was downregulated.<sup>71</sup> This change could lead to an increase in iron accumulation within the cells.<sup>71</sup> Despite the augmented apoptosis and the upregulated levels of MMP3 and MMP13 in chondrocytes treated with Ferric Ammonium Citrate (FAC), which establish a connection between iron



**Figure 1** The impacts of iron overload and lipid peroxidation on OA during ferroptosis. Ferroptosis is initiated due to excessive lipid peroxidation (LPO). The regulation of the efficacy of cysteine and reduced glutathione (GSH) is carried out by the cystine-glutamate reverse transport system Xc- or the transsulfuration pathway. The transsulfuration route is a metabolic way which converts cysteine into homocysteine, generating amino acids containing cysteine (Cys) and synthesizing GSH. Glutamine can be transported into the cell via the SLC1A5 transporter protein and subsequently transformed into glutamate by glutamine synthetase (GLS), thereby influencing the utilization rate of GSH. GSH serves as a crucial cofactor for GPX4 in eliminating lipid peroxides. On the one hand, it is believed that the oxidative effects of polyunsaturated fatty acids and excess iron constitute an important factor. The uptake of iron through transferrin receptor 1 (TFR1) or the degradation of ferritin iron reserves can enhance an unstable iron pool, therefore, cells are susceptible to ferroptosis due to the production of lipid hydroperoxides by the Fenton reaction. Ferroptosis can gradually intensify inflammatory responses, resulting in the augmented expression of enzymes like MMP-13 in chondrocytes and the reduced expression of collagen II, thereby accelerating the advancement of OA. Created in BioRender. Jiwei, H (2025) <https://BioRender.com/9n3xifo>.

**Abbreviations:** POR, Cytochrome P450 reductase; LOX, Lipoygenase; STEAP3, Prostate-specific Membrane Antigen 3; DMT1, Divalent metal transporter 1; Glu, Glutamate; Cys, Cysteine; GCL, Glutamylcysteine ligase; Gly, Glycine; GSH, Glutathione; GPX4, Glutathione peroxidase 4; Gln, Glutamine; SLC1A5, Transport protein; Gls, Glutaminase; PUFAs, Polyunsaturated fatty acids.

overload and chondrocyte apoptosis, this might disclose the role of chondrocyte ferroptosis in OA.<sup>71</sup> In individuals with OA, synovial cells produce 4-HNE (4-hydroxyheptenaldehyde), a byproduct from the oxidative degradation of polyunsaturated fatty acids, alongside interleukins and chemokines which play a significant role in the inflammatory process. Furthermore, there is an increase in the concentration of 4-HNE protein adducts present in both synovial fluid and osteoblasts.<sup>74–76</sup> Studies have shown that high levels of 4-HNE are detrimental to human chondrocytes and

osteoblasts.<sup>75–77</sup> Research has shown that mitochondrial DNA damage in OA negatively affects chondrocytes.<sup>78</sup> Studies show that ferroptosis in chondrocytes can play a major role in the advancement of OA.<sup>79</sup> Yao and his team utilized IL-1b to mimic an inflammatory response and employed fluorescence-activated cell sorting (FAC) technology to replicate the conditions of iron overload in chondrocytes.<sup>79</sup> In murine knee joint chondrocytes after simulating inflammation with interleukin-1 beta (IL-1 $\beta$ ) and iron overload with FAC in vitro, the buildup of ROS and lipid peroxides, along with alterations in apoptosis-related proteins like GPX4, SLC7A11, ACSL4, P53, and NRF2ARE, can trigger ferroptosis.<sup>79</sup> Conversely, Fer-1, which is a known inhibitor of iron-induced ferroptosis, can help diminish this process. In mouse chondrocytes, exposure to Erastin, known to trigger ferroptosis, caused an increase in MMP13 expression in cells, while also reducing the levels of type II collagen.<sup>79</sup> Both of these markers are linked to OA. The results were additionally validated in OA mouse models that received treatment with FER-1.<sup>79</sup> In the OA mouse model, there is a direct correlation between the levels of iron in synovial fluid and the advancement of OA.<sup>80</sup> Excessive iron levels in the body can cause synovitis and hyperplasia, result in the death of chondrocyte cells, and impair the function of subchondral osteogenic cells.<sup>73</sup> Research has indicated that excessive iron can disturb the iron balance in chondrocytes, resulting in oxidative stress and eventually triggering cell apoptosis.<sup>81</sup> As a result, it is essential to maintain strict monitoring of the iron levels in the organization.<sup>82</sup> Studies show that rats with iron overload had higher microCTOA and OARSI histological scores in their knee joints than those in a control group.<sup>71</sup> This implies that excess iron may play a role in worsening the pathological damage linked to OA. Additional analysis indicated that rats exposed to the iron overload model exhibited markedly elevated levels of iron and mRNA expression for FTH-1, FPN, IL-1 $\beta$ , and TNF in both the cartilage of the knee and the fat pad of the patella.<sup>83</sup> Conversely, there was a significant reduction in the mRNA levels of TFR, DMT1, Col2, and aggrecan. Research indicates that excess iron is linked to increased ferritin levels, heightened generation of inflammatory cytokines, and a reduction in the synthesis of cartilage tissue.<sup>84</sup> The findings indicate that a high level of iron could play a major role in the onset of OA. In conclusion, an excess of iron can trigger the production of ROS and pro-inflammatory cytokines in chondrocytes. This process results in the breakdown of cartilage tissue and hinders its formation, leading to joint cartilage deformities and worsening the advancement of OA.<sup>85</sup> **Figure 1.**

#### Lipid Peroxidation and Osteoarthritis

When ROS target the lipids in cell membranes, they generate peroxidized lipids like malondialdehyde (MDA). These compounds can interact with DNA or proteins within the organism, resulting in additional harm to the cell membrane's structure and composition.<sup>86,87</sup> Research indicates that in a rat model of OA induced by anterior cruciate ligament transection (ACLT) surgery, there is an increase in serum levels of MDA and lipid peroxides. Furthermore, reducing the concentrations of MDA and lipid peroxides may help slow the progression of OA.<sup>88</sup> GPX4 is an enzyme that plays a role in repairing lipids and is involved in the oxidation of liposomes, with its activity being affected by GSH.<sup>89</sup> Studies have shown that in regions of OA lesions with increased lipid peroxidation, levels of GPX4, GSH, and the GSH to GSSG ratio in chondrocytes are significantly lower. This implies that lipid peroxidation takes place as OA progresses.<sup>90</sup> In a different in vitro experiment designed to cause oxidative damage to cells using a similar concentration of tert-butyl hydroperoxide (TBHP), researchers found that reducing GPX4 levels in mouse chondrocytes (ATDC5) and primary mouse chondrocytes (MCC) made the cells more susceptible to oxidative stress, resulting in a significant rise in MDA levels.<sup>91,92</sup> The findings suggest that lower levels of GPX4 expression make chondrocytes more vulnerable to oxidative stress by elevating lipid peroxidation in these cells.<sup>90</sup> As a result, GPX4 could be an important target for affecting lipid peroxidation in relation to OA. **Figure 1.**

#### The Xc–system/GSH/GPX4 Regulatory Axis and Osteoarthritis

The regulatory pathway involving Xc-system/GSH/GPX4 is crucial for preventing ferroptosis in articular chondrocytes. Wang<sup>93</sup> and his team analyzed cartilage samples taken from the loading (Acronym L) area and unloading (Acronym UL) area of patients with OA who had total knee arthroplasty (Acronym TKA). The researchers utilized transmission electron microscopy to investigate the changes in the shape of chondrocytes from the L-zone of osteoarthritic cartilage linked to ferroptosis, which included thickening of the mitochondrial membrane and contraction of the mitochondria.<sup>94</sup> Furthermore, the findings from the mRNA microarray analysis show that the expression of the GPX4 biomarker associated with ferroptosis is lower in chondrocytes located in the loading region than in those in the unloading

region.<sup>93</sup> At the same time, research conducted on cells revealed that primary mouse chondrocytes subjected to a mechanical stress of 1 MPa exhibited alterations in their mitochondrial structure associated with iron imbalances, in contrast to those kept without any mechanical stress.<sup>95</sup> The findings indicate that mechanical stimulation results in reduced levels of GPX4 mRNA and protein expression. The *in vivo* study provided additional evidence showing that the levels of GPX4 protein in the cartilage of mice with an OA model were notably reduced.<sup>93</sup> The research findings suggest that GPX4 plays a crucial role in regulating ferroptosis in chondrocytes and cartilage cells located in the weight-bearing regions of joint cartilage in OA patients experiencing high mechanical stress. The level of GPX4 expression might be linked to the advancement of OA.<sup>93</sup> Exosomes from osteoarthritic fibroblast-like synoviocytes promote cartilage ferroptosis and damage via delivering microRNA-19b-3p to target SLC7A11 in OA.<sup>96</sup> The Xc-system's precise control of SLC7A11 is crucial for the accumulation of iron storage proteins in OA.<sup>97</sup> *In vitro* studies provide additional evidence that excess iron enhances the production of ROS and lipid peroxides in chondrocytes, while reducing the levels of Xc-system (xCT), GSH, GPX4, and the mitochondrial membrane potential in these cells. Consequently, the regulatory pathway involving Xc-system/GSH/GPX4 is crucial for managing iron-related mutations associated with OA.<sup>98</sup>

## Pyroptosis and Osteoarthritis

### The Molecular Mechanism of Pyroptosis

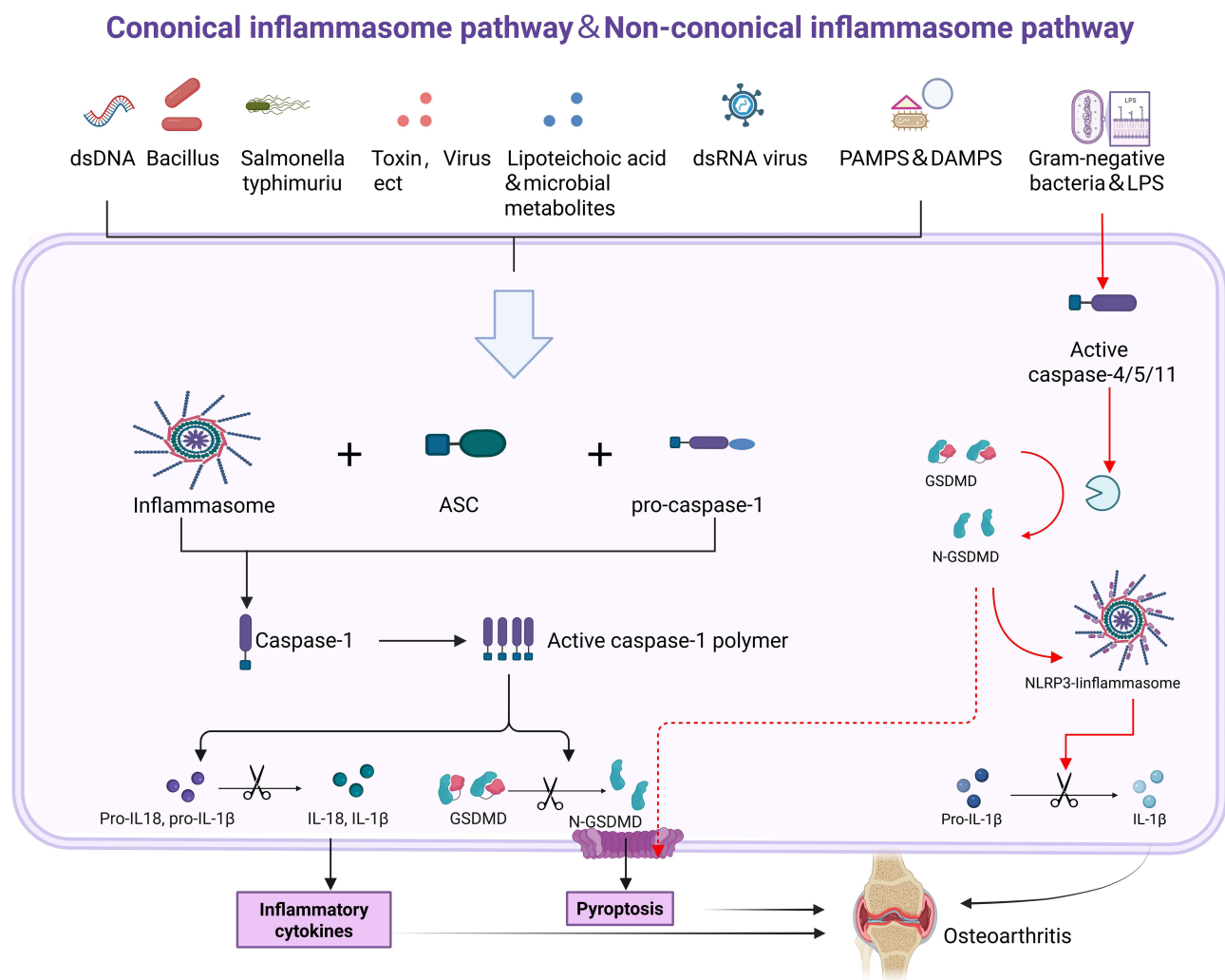
Cell pyroptosis, often referred to as inflammatory necrosis, is a controlled form of PCD that relies on the Gasdermin protein family to create pore-like formations in the plasma membrane.<sup>99</sup> This process entails the gradual swelling of the cell until the membrane breaks, leading to the release of internal substances and the activation of an inflammatory response.<sup>100</sup> Inflammasomes are protein complexes found in the cytoplasm, usually made up of sensors (PRRs), adapters (ASC), and effectors (caspase-1).<sup>101</sup> Sensors are receptors for pattern recognition (PRRs) that mainly consist of nucleotide-binding oligomerization domain (NOD) and leucine-rich repeat (LRR) receptors (NLRs), AIM2-like receptors (ALR), and pyrin (protein interaction modules).<sup>102</sup> Caspases that play a role in pyroptosis are commonly known as pro-inflammatory caspases. This group typically consists of human caspases 1, 4, and 5, as well as mouse caspase 11.<sup>103</sup> Gasdermins are a newly identified family of proteins known for their ability to form pores in cell membranes, with gasdermin D (GSDMD) playing a central role in the process of pyroptosis.<sup>104</sup> Certain caspases or granzymes trigger the activation of the protein's N-terminal region, which reinstates its cytotoxic abilities and encourages the secretion of inflammatory substances and cytokines.<sup>105</sup> At present, the mechanisms of pyroptosis can be broadly categorized into three groups: the standard inflammasome pathways that rely on caspase-1, the non-standard inflammasome pathways that depend on caspases-4/5/11, and additional pathways that may or may not involve caspases.<sup>106</sup> The activation of intracellular receptors such as NLRP1, NLRP3, NLRP6, NLRP9, and NLRC4 by certain pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) is critical, as these processes are known to contribute to inflammatory responses.<sup>107</sup> In the context of OA, this activation is implicated in exacerbating inflammatory pathways that contribute to joint degradation and disease progression.<sup>108</sup> An example of this is the harmful substance produced by *Bacillus anthracis*, which can activate NLRP1b in mice.<sup>109</sup> The activation of NLRP6/9 can take place as a reaction to viral or bacterial infections during the innate immune response.<sup>110,111</sup> In contrast, NLRP3 is the intracellular sensor that has been studied the most extensively, NLRP3 is capable of reacting to infections caused by bacteria, viruses, and fungi, in addition to endogenous DAMPs that trigger sterile inflammation and various environmental factors, this is what sets it apart from the majority of PRRs that focus on just one or a limited number of PAMPs or DAMPs.<sup>112</sup> It is important to highlight that the full activation of NLRP3 occurs in two steps: the initiation phase and then the activation phase. The first step in activation is the transcription of NLRP3, which requires the involvement of Toll-like receptors (TLRs), interleukin-1 receptors, and tumor necrosis factor receptors.<sup>113</sup> As a result, the signals that are sent regulate NLRP3, procaspase-1 $\beta$ , and procaspase-18 via the NF- $\kappa$ B pathway. Furthermore, when the NAIP protein interacts with triggers like *Salmonella enterica* serovar Typhimurium, it activates NLRC4, leading to an inflammatory response.<sup>114</sup> Besides NLRs, there are other intracellular sensors such as pyrin and AIM2 that primarily serve a function in detecting viral infections and dangerous pathogens. As a result, the activated pattern recognition receptors (PRRs) engage with each other via CARD-CARD domains, which leads to the recruitment of ASC (apoptosis-associated speck-like protein) and the binding of procaspase-1, ultimately forming the inflammasome.<sup>115</sup> In response to transmitted danger signals, procaspase-1 is cleaved and activated, resulting in the formation of caspase-1 polymers known as p10/p20 tetramers. The active version of Caspase-1 is

capable of identifying pro-IL-1 $\beta$  and pro-IL-18, even though these precursors lack biological activity.<sup>103</sup> On the other hand, when caspase-1 is activated, it can cut GSDMD, which is crucial for initiating cell pyroptosis. This cleavage leads to the creation of openings in GSDMD, promoting cell pyroptosis and facilitating the release of biologically active substances, this encompasses IL-1 $\beta$ , IL-18, and high mobility group box 1 protein (HMGB1).<sup>104</sup> Figure 2.

## The Role of Pyroptosis in Osteoarthritis

### Chondrocyte Pyroptosis

It remains uncertain whether cartilage deterioration in OA is a contributing factor or a consequence of the disease, numerous research studies have indicated that various risk factors associated with OA, such as oxidized low-density lipoprotein (Ox-LDL), cholesterol related to obesity, hydroxyapatite crystals, and issues with intestinal circulation that result in the movement of lipopolysaccharides (LPS), can initiate pyroptosis via molecules associated with damage (DAMP) or molecules associated with pathogens (PAMP).<sup>116–118</sup> These substances trigger pyroptosis in chondrocytes via the NLRP3 inflammasome pathway, interfere with the metabolism of different compounds, and lead to the release of important molecules that promote the advancement of OA. Key molecules involved include matrix metalloproteinases (MMPs), cysteine proteases with propeptide sequences (like cathepsins), ADAMTS metalloproteinases, and cytokines including IL-1 $\beta$ , IL-18, and TNF- $\alpha$ .<sup>119,120</sup> Studies have shown that MMP-1, MMP-3, MMP-13, and ADAMTS-4/5 are



**Figure 2** The molecular mechanism of pyroptosis and its impact on OA. Typical inflammatory pathways (black arrows). Atypical inflammatory body pathway (red arrow). Pyroptosis is mainly induced by the NLRP3 inflammasome. The NLRP3 inflammasome is capable of activating GSDMD or triggering the generation of IL-1 $\beta$  and IL-18, thereby intensifying the progression of OA. Created in BioRender. Jiwei, H (2025) <https://BioRender.com/v25o168>.

the primary agents engaged in the disintegration of cartilage ECM components in the mentioned molecules,<sup>121,122</sup> The rise in their levels can be somewhat attributed to the secretion of IL-1 $\beta$  and TNF- $\alpha$  from cartilage cells undergoing pyroptosis.<sup>123,124</sup> As a result, the incidence of pyroptosis in chondrocytes disturbs the equilibrium between the synthesis and breakdown of the ECM in chondrocytes, causing a shift towards increased degradation metabolism as a consequence of chondrocyte pyroptosis. Furthermore, the released IL-1 $\beta$ , IL-18, and TNF- $\alpha$  disseminate to the cartilage, synovial fluid, and even the subchondral bone, leading to additional harm to the joints affected by OA.<sup>125</sup> IL-1 $\beta$  could be associated with the breakdown of cartilage and the inflammatory process. The activities related to IL-1 $\beta$  in chondrocytes encompass gene expression, cell growth, and cell apoptosis. IL-1 $\beta$  influences the expression of MMP-1 and MMP-11,<sup>123</sup> and it also triggers the generation of ROS,<sup>126</sup> In the end, it leads to the death of chondrocytes and the breakdown of the ECM, creating a harmful cycle of positive feedback. When the feedback loop speeds up the breakdown of cartilage, pieces of cartilage may be released into the synovial fluid, leading to additional harm to the synovial membrane.<sup>127</sup> Moreover, the presence of IL-1 $\beta$  in synovial fluid encourages the production of inflammatory substances as well as various cytokines and chemokines, this includes inducible nitric oxide synthase (iNOS),<sup>127</sup> Cyclooxygenase-2 (COX-2),<sup>128</sup> IL-6,<sup>129</sup> IL-8<sup>130</sup> and CCL5,<sup>131</sup> consequently, it plays a role in controlling inflammation in the synovial tissue. The biological impacts of TNF- $\alpha$  resemble those of IL-1 $\beta$ , and they have the ability to stimulate one another.<sup>132</sup> TNF- $\alpha$  has the ability to elevate levels of inflammatory substances like PGE2 and VEGF, which subsequently enhances inflammatory reactions and promotes vascular infiltration.<sup>133,134</sup> While IL-18 can trigger the synthesis of MMPs and lead to chondrocyte pyroptosis, these events are more likely to happen via pathways that depend on IL-1 and/or TNF- $\alpha$ .<sup>135,136</sup> In general, the immediate consequence of chondrocyte apoptosis in articular cartilage is the removal of chondrocytes, which disturbs the previously stable architecture of the cartilage ECM and leads to the release of pro-inflammatory substances. This process indirectly speeds up cartilage deterioration and initiates joint inflammation. Consequently, treatments targeting IL-1 $\beta$  and TNF- $\alpha$  might remain some of the limited effective approaches for reducing chondrocyte pyroptosis, lessening the degradation of the ECM, and easing inflammatory reactions.

### Synovial Cell Pyroptosis

Research indicates that alterations in the synovial membrane of joints affected by OA can be classified into three main categories: accumulation of debris, inflammatory responses, and fibrosis.<sup>137</sup> Nonetheless, synovial macrophages and fibroblast-like synoviocytes (FLS) are significantly associated with the alterations occurring in the synovium of joints affected by OA.<sup>138</sup> As previously stated, the components found in the synovial fluid consist of: fragments of degenerated cartilage, cytokines that are released, and proteases produced by dying chondrocytes. They function as DAMPs by engaging with the membrane TLRs found in synovial macrophages and FLS, which initiates pyroptosis and leads to harmful alterations in the synovial tissue.<sup>138,139</sup> Certain studies indicate that chondrocytes in OA communicate with synovial cells via vesicles resembling exosomes.<sup>140</sup> Regardless of the previously mentioned factors, MMPs, ADAMTS, IL-1 $\beta$ , IL-18, and TNF- $\alpha$  worsen cartilage damage and synovitis. During pyroptosis, synovial macrophages and FLS continue to generate high mobility group box 1 (HMGB1) and transforming growth TGF- $\beta$ .<sup>141,142</sup> HMGB1 engages with FLS and facilitates the clustering of pro-inflammatory substances, which contributes to the onset of synovitis,<sup>143</sup> HMGB1 has the ability to attach to LPS and be carried to synovial macrophages via RNA, leading to pyroptosis.<sup>144</sup> TGF- $\beta$  elevated the levels of expression of fibrosis markers in OA FLS, for instance, the expression levels of collagen lysine, prolyl 4-hydroxylase 2 (PLOD2), type I collagen alpha 1 chain (COL1A1), and tissue inhibitor of metalloproteinases 1 (TIMP1) are being examined, this could ultimately cause an excess production of ECM, resulting in fibrosis.<sup>141</sup> Moreover, the injured synovium triggers the production of HIF-1 $\alpha$  and ATP, which prompts FLS to experience pyroptosis, resulting in synovitis and fibrosis.<sup>145,146</sup> In summary, recent research indicates that the pyroptotic mechanism in synovial cells and the particular cytokines they generate are linked to the synovitis and fibrosis seen in OA.<sup>141</sup> Simultaneously, the loss of cartilage and the development of fibrosis affect chondrocytes via cytokines, resulting in the depletion of cartilage and alterations in the synovial tissue. This establishes a harmful positive feedback loop akin to the process of cartilage degradation.<sup>147</sup> Most synovial macrophages are classified as M2 type, and they play a role in managing inflammation when they are activated. M2 macrophages have the ability to convert into M1 macrophages, leading to an inflammatory reaction. In people with OA, macrophages accumulate crystals in the synovial tissue.<sup>148</sup>

When a cell dies, ATP is released, triggering the triggering of the NLRP3 inflammasome, which leads to the production of IL-1 $\beta$  and IL-18.<sup>130</sup> The existence of sodium urate crystals in the joints is linked to the severity of OA and the decline of bone health. Lowering the levels of these crystals could help maintain joint function. Caspase-1 is essential for creating pores in GSDMD, which intensifies inflammation in the synovial tissue. Zhang and his team noted a rise in the components of the NLRP3 inflammasome found in the synovial tissue of rats with OA of the knee joint.<sup>149</sup> Employing a caspase-1 inhibitor may enhance tissue structure, lower inflammation, reduce fibrosis, and decrease the amounts of pro-inflammatory substances. Nonetheless, inhibiting caspase-1 can decrease pyroptosis triggered by LPS, although the macrophages do not originate directly from the affected synovium.<sup>112</sup> In line with the earlier statement, FLS cells displayed characteristics of pyroptosis and demonstrated elevated levels of fibrotic factors when co-cultured with macrophages undergoing pyroptosis.<sup>122</sup> It is evident that macrophage pyroptosis contributes to the progression of OA synovium. Nevertheless, further experiments are required to investigate the various connections between the two, since in the advanced stages of OA synovium, pyroptosis is frequently linked to catabolic processes, whereas fibrosis is mainly associated with anabolic processes. The change in metabolic activity indicates that a broader variety of cells or cytokines may be participating in the process.

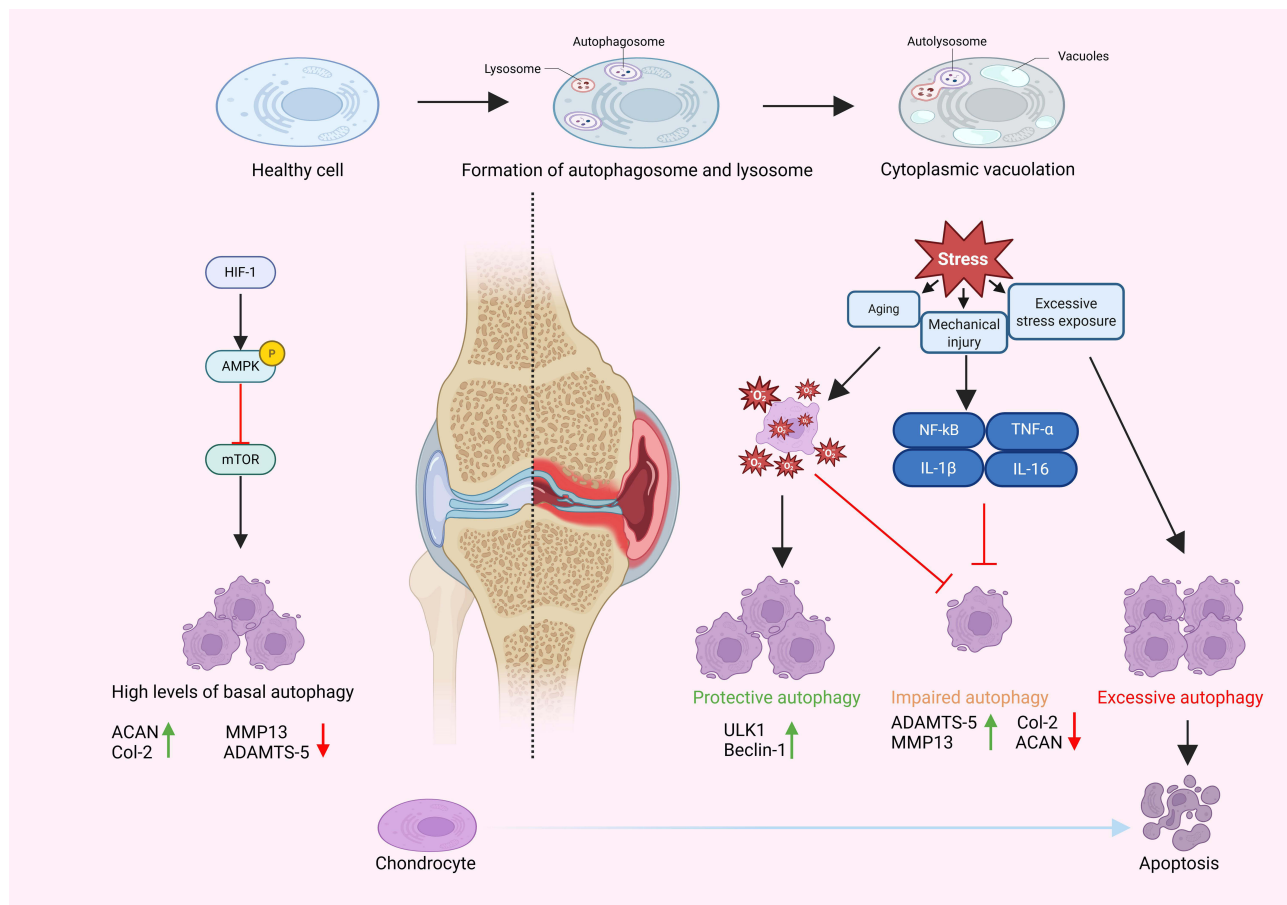
### Pyroptosis in Subchondral Bone

Subchondral bone, an essential part of the knee joint's composition and function, is a thin layer of dense bone situated beneath the calcified cartilage. In patients with OA, the thickness of the subchondral cortical bone is impacted, resulting in subchondral sclerosis, excess bone formation, bone spurs, and the development of bone cysts in later stages.<sup>150,151</sup> When the inner meniscus (DMM) model is unstable, a rise in pyroptosis-related molecules results in a notable increase in the OARSI (Osteoarthritis Research Society International) score, Yan<sup>152</sup> et al identified the effects of pyroptosis in the OA model, observing an increase in the levels of pyroptosis-related factors (including NLRP3, Caspase-1, GSDMD, and IL-1 $\beta$ ) in chondrocytes, which corresponded with a rise in the OARIS score. During the early phase of the research, they noted a reduction in the mass of subchondral bone, an increase in trabecular separation (Tb.Sp), a rise in osteoclast numbers, and a decline in osteoprotegerin (OPG) levels.<sup>152</sup> In the advanced stages of OA, they noted a rise in subchondral bone volume along with a reduction in trabecular bone (Tb).<sup>152</sup> The results suggest a strong relationship between chondrocyte pyroptosis and alterations in subchondral bone during the progression of OA.

## Autophagy and Osteoarthritis

### The Molecular Mechanisms of Autophagy

Autophagy is a process that recycles cellular waste by encapsulating aging cells, damaged organelles, misfolded proteins, and other materials into autophagosomes, which are subsequently sent to lysosomes for breakdown.<sup>153</sup> This mechanism plays a crucial role in sustaining energy balance, allowing the body to cope with stressors like nutrient shortages, low oxygen levels, and other challenges.<sup>154,155</sup> Autophagy involves several important stages, such as the start of the process, the formation of vesicles (creating a double-membrane isolation membrane), the growth and development of these vesicles, the merging of the autophagosome with the lysosome, and the degradation of the autophagosome.<sup>156–159</sup> **Figure 3.** Autophagy starts with the ULK1 complex, which is made up of ULK1, ATG13 (a protein associated with autophagy), the 200kDa FIP200 protein that interacts with proteins from the adhesion-related kinase family, and ATG101. Once autophagy begins, the ULK1 complex detaches from the mTOR complex and moves to the location where autophagy starts. At this site, it phosphorylates ATG13 and FIP200, thereby triggering the autophagy process.<sup>160</sup> Subsequently, the ULK1 complex brings in the Beclin1-Vps34 complex, which consists of Beclin1, Vps34, Vps15, and ATG14L, to the precursor structure of the autophagosome by phosphorylating Ambra1, a protein that plays a role in vesicle formation.<sup>161</sup> During this process, Ambra1 interacts with the tumor necrosis factor receptor-associated factor (TRAF6) and functions as the E3 ubiquitin ligase for ULK1, adding K63 ubiquitin chains and enhancing the stability of ULK1. The activation of ULK1 can result in the phosphorylation of Beclin1, thereby increasing the activity of the Vps34 complex.<sup>162</sup> Once the ULK1 and Beclin1-Vps34 complex is activated, the phosphorylation of downstream targets commences. This results in an increase in phosphatidylinositol-3-phosphate at the site of autophagosome formation, which is vital for recruiting proteins necessary for the development of autophagosomes. For instance, the WD-repeat



**Figure 3** The general process of autophagy and its role in cartilage and chondrocytes. In regular joint cartilage, HIF-1/AMPK/mTOR constitutes the primary pathway for the activation of autophagy in cartilage cells under hypoxic circumstances. Moreover, AMPK restrains the activation of mTOR, while mTOR hinders autophagy. At the initial phases of OA, autophagy temporarily increases in reaction to pathogenic elements like mechanical injury and aging, which cause oxidative stress. In the late stage of OA, the levels of oxidative stress and inflammation rise further, exceeding the repair capacity of autophagy, this brings about a reduction in autophagy, leading to an imbalance in the synthesis and degradation of cartilage. Furthermore, in cases where chondrocytes are overly exposed to stress stimuli, excessive autophagy might take place, which further intensifies chondrocyte apoptosis. Created in BioRender. Jiwei, H (2025) <https://BioRender.com/lis3jwl>.

domain phosphatidylinositol (PI) interaction protein and DFCP1 are involved in this process. This results in the formation and growth of autophagosomes, ultimately leading to the creation of fully developed autophagosomes.<sup>163,164</sup>

The process of elongating and maturing autophagosomes requires two systems that involve ubiquitin-like conjugation: the ATG12-ATG5-ATG16L complex and LC3-phosphatidylethanolamine.<sup>165</sup> ATG12, in collaboration with ATG7 and ATG10, attaches LC3 to phosphatidylethanolamine (PE). ATG10 interacts with ATG5, which then associates with ATG16L to create the ATG12-ATG5-ATG16L complex.<sup>166</sup> LC3 is processed by ATG4 to create LC3-i, which is subsequently linked to PE via the ATG3-ATG7 and ATG5-ATG12 complexes, resulting in the formation of LC3-ii. This version of LC3 attaches to the autophagosome and plays a role in its maturation.<sup>167</sup> Once autophagosomes are formed, they travel along microtubules to the area near the lysosomes surrounding the nucleus and establish contact with them.<sup>168</sup> Using the Rab-SNARE system along with various autophagy proteins, the outer membrane of the autophagosome merges with the lysosomal membrane. This fusion allows the release of degradation enzymes into the fully formed autolysosome, which then break down the contents.<sup>169,170</sup>

### The Role of Autophagy in Osteoarthritis

Autophagy plays a crucial role in regulating the survival and lifecycle of chondrocytes by maintaining cellular homeostasis.<sup>171</sup> Nonetheless, certain research indicates that too much autophagy could result in damage and death of cartilage cells.<sup>172</sup> Next, we will concentrate on the dual function of autophagy in cartilage or chondrocytes. [Figure 3](#).

## Protective Autophagy in Chondrocytes and Cartilage

**Autophagy and Hypoxia.** OA is a condition that is not related to blood vessel inflammation, in which the joint cartilage exists in a low-oxygen setting for its entire life. It depends on HIF-1 and HIF-2 to adjust to this lack of oxygen. HIF-1 is found in both healthy cartilage and cartilage affected by OA.<sup>173</sup> It supports the growth of healthy cartilage by enhancing the characteristics of cartilage cells, their survival, and the production of the cartilage matrix. HIF-2 is responsible for facilitating the metabolic process that results in the breakdown of the OA cartilage matrix, which ultimately causes damage to the joint cartilage.<sup>174</sup> In contrast to cells that thrive in oxygen-abundant conditions, cartilage cells mainly depend on autophagy instead of cell turnover to preserve a healthy cartilage endothelium.<sup>175</sup> In an environment lacking oxygen, chondrocytes show increased basal autophagy and generate a higher quantity of col-2 cells, leading to stronger collagen structures in the joint.<sup>176</sup> On the contrary, inhibiting HIF-1 would negatively impact cell autophagy, resulting in the buildup of MMP13 and impairing mitochondrial function.<sup>177</sup> The primary pathway for triggering autophagy in chondrocytes during low oxygen conditions is the HIF-1-adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK)-mTOR pathway.<sup>178</sup> Under hypoxic conditions, cellular energy levels drop, prompting the activation of HIF-1, which enhances glycolysis. This process results in increased phosphorylation of AMPK, which subsequently suppresses mTOR activity, ultimately leading to the onset of autophagy.<sup>179</sup> This guarantees that chondrocytes stay alive and finish their life cycle in their designated low-oxygen environment following cell division.<sup>178</sup> At the same time, HIF-2 suppresses autophagy. When HIF-2 was removed, there was a notable increase in the autophagy response in the chondrocytes of the growth plate cartilage in mice. This increase was linked to a decrease in the activity of protein kinase B (Akt), mTOR, and the expression of B cell lymphoma-2 (Bcl-2).<sup>180</sup>

**Autophagy and Aging.** Autophagy is involved in the development and final differentiation of the growth plate in hypertrophic chondrocytes.<sup>181</sup> The temporary rise in the levels of ULK1, Beclin1, and LC3 suggests that autophagy may serve as a protective response to cellular stress in the initial degenerative phase of OA. However, as the pathological changes advance to mild OA, the amount of autophagy proteins in the surface of the joint cartilage diminishes. In the advanced phase of OA, there are defects in autophagy across the entire articular cartilage layer, affecting both the middle and deeper regions, which eventually results in the death of chondrocytes.<sup>182,183</sup> Chondrocyte autophagy takes place before cartilage damage happens, and its function gradually declines with age, which aligns with the deterioration of chondrocytes.<sup>184</sup> Additionally, a recent study indicates that the progression of OA deteriorates with the aging of fibroblast-like synovial cells, which is associated with reduced autophagy and increased levels of the senescence-associated secretory phenotype (SASP). The restoration of autophagy counteracts the effects of aging and the secretion of the SASP, while also reducing cartilage damage by limiting GATA4 activity.<sup>185</sup> The data indicates that autophagy serves as a protective mechanism for joint cartilage and synovial tissue in response to different stress conditions. However, prolonged exposure to the damage caused by excessive stress that exceeds the repair capacity of autophagy can worsen cartilage degeneration.<sup>184</sup> OA worsens as people age, and this may be linked to apoptosis that depends on caspases.<sup>186</sup> In GFP-LC3 transgenic mice, LC3 and ATG5 are most highly expressed in the chondrocytes found in the superficial and middle layers. Additionally, the removal of mouse ATG5 results in the early aging of these chondrocytes. In older mice, there is a notable decrease in the quantity of autophagosomes within chondrocytes, and the amount of vesicles in the subchondral bone declines significantly. Dysfunctional autophagy worsens OA-like alterations in the synovial tissue.<sup>187</sup> In healthy adults, significant autophagy is present in the outer layer of cartilage, while cartilage in older individuals exhibits reduced autophagy levels.<sup>182</sup> This suggests that impaired autophagy contributes to premature aging in cartilage, which in turn causes irregular cartilage production, abnormal expression of catabolic genes, and damage to the ECM. In addition, mechanical injury to cartilage can influence the autophagy of cartilage cells. Research has indicated that mechanical injury can suppress autophagy.<sup>188</sup> This is associated with a notable reduction in the expression of ULK1, Beclin1, and LC3 in the outer region of the cartilage within 48 hours post-injury, whereas the stimulation of external autophagy can restore cartilage damage resulting from mechanical injury.<sup>188</sup> In contrast, suitable mechanical stress can help reduce aging and inflammation in chondrocytes, manage autophagy, improve chondrocyte function, and lessen cartilage deterioration.<sup>189,190</sup> In a mouse model of OA, suitable mechanical stress boosted autophagy

in cartilage, leading to higher levels of LC3 and lower levels of p62/SQSTM1. This ultimately facilitated the formation of cartilage cells and improved the recovery from cartilage damage.<sup>191</sup>

**Autophagy and Inflammation.** The levels of inflammatory factors in the synovial tissue and subchondral bone are closely linked to the degenerative alterations in the structure of cartilage affected by OA.<sup>27</sup> Research indicates that these inflammatory factors can speed up the aging process of cartilage cells by hindering autophagy and triggering inflammatory reactions, which in turn worsens OA.<sup>192</sup> The inflammatory response triggered by IL-1 $\beta$  significantly increases the phosphorylation of PI3K, Akt, and mTOR proteins, resulting in a reduction of autophagy activity, this results in an obstruction of the G1 phase in chondrocytes and a decrease in the S phase. Additionally, inhibiting the PI3K-Akt-mTOR pathway can enhance autophagy in chondrocytes of OA rats and reduce inflammatory reactions.<sup>193</sup> In OA mice, the targeted reduction of PPAR $\gamma$  results in an increased generation of inflammatory markers such as inducible nitric oxide synthase and cyclooxygenase-2. This is linked to the heightened expression of mTOR and a decrease in important autophagy markers, which in turn intensifies the inflammatory response in the cartilage and accelerates the development of OA.<sup>194</sup> The mTOR-mediated autophagy pathway is significantly linked to synovitis in OA. Inhibiting mTOR triggers the activation of autophagy, which in turn decreases the formation of synovial osteoclasts and reduces IL-1 expression in joint cartilage, thereby safeguarding the cartilage from erosion.<sup>195</sup> The activation of the MAPK/NF- $\kappa$ b pathway is advantageous for reducing autophagy levels in kidney cells during OA.<sup>196</sup> Recent studies have revealed that epigenetic changes play a role in the connection between autophagy and inflammation in patients with OA.<sup>197</sup> Methyltransferase-like 3 enhances the stability of Bcl-2 mRNA via the M6A/Yth m6A RNA binding protein 1 pathway, this limits the autophagy process driven by Beclin1 in the body, which in turn increases the apoptosis of cartilage cells and the deterioration of subchondral bone in mice with OA.<sup>197</sup>

**Autophagy and Oxidative Stress.** Oxidative stress arises when there is an imbalance between the generation of ROS in the mitochondria and the capacity to eliminate them.<sup>198</sup> In OA chondrocytes, different factors like mechanical damage and aging trigger the activation of cytokine receptors and Toll-like receptors (TRL), which in turn increases the production of superoxide (O<sup>-</sup> 2) in the mitochondria and decreases the levels of antioxidant enzymes superoxide dismutase 1 and superoxide dismutase 2, this eventually results in the buildup of ROS and the impairment of mitochondrial function.<sup>139,199</sup> The oxidative alteration of both intracellular and extracellular elements, along with the suppression of collagen production and the activation of the MMP and ADAMTS families, contributes to the deterioration and breakdown of cartilage.<sup>200</sup> The breakdown products continue to worsen joint deterioration, creating a harmful cycle.<sup>201</sup> During the early stages, the autophagy process in OA cartilage is increased, but it diminishes in the later stages. This indicates that autophagy may serve as a protective mechanism against oxidative stress. As a result, some individuals propose that enhancing autophagy could aid in eliminating the excessive buildup of inflammatory substances and ROS.<sup>202</sup> The excessive buildup of p62 in OA chondrocytes due to oxidative stress results in disrupted autophagy and heightened chondrocyte apoptosis. On the other hand, treatment with alginate specifically addresses the autophagic damage caused by oxidative stress and promotes autophagy by activating Bcl2 interacting protein 3 (BNIP3).<sup>203</sup>

### Harmful Autophagy in Chondrocytes and Cartilage

The studies mentioned indicate that in models of aging or OA, the autophagy function in joint cartilage or chondrocytes is compromised. Restoring proper autophagy levels may help mitigate the pathological damage resulting from inflammation and oxidative stress in OA, ultimately leading to a reduction in the condition.<sup>172,204,205</sup> However, an over-activation of autophagy could negatively impact the regulation of OA. Mitofusin2 is an important molecule that plays a role in controlling autophagy and inflammation. When it is overexpressed, it can greatly suppress the PI3K/AKT/mTOR pathway, leading to an overproduction of autophagy. This disrupts the normal balance of autophagy in chondrocytes, raises the levels of ROS, and triggers inflammatory responses, ultimately resulting in cartilage degeneration.<sup>206</sup> Chang<sup>172</sup> et al discovered that the autophagy levels in the cartilage of OA patients are considerably greater than those in young injured cartilage or in cartilage from older individuals. The cell clusters in the upper region of the cartilage show a strong expression of Beclin-1 and LC3, and there is evidence of autophagic cell death occurring in the chondrocytes of OA patients. This may be linked to an overactivation of autophagy.<sup>172</sup> When chondrocytes are grown in an acidic environment for 48 hours, the acid-sensing ion channel 1a, which is activated by the external acidity, triggers

chondrocyte senescence by continuously activating autophagy. This indicates that consistently elevated autophagy levels might contribute negatively to the senescence of OA chondrocytes.<sup>154,207</sup> Moreover, oxidative damage to DNA can result in overactivation and improper regulation of autophagy. When healthy cartilage cells experience acute external oxidative stress, the expression of autophagy-related genes rises initially but returns to normal within 6 to 24 hours.<sup>208</sup> In contrast, the autophagy levels in OA cartilage cells stay elevated for an additional 24 hours.<sup>209</sup> In OA chondrocytes, oxidative stress causes mitochondrial impairment, leading to a higher generation of ROS. This ROS serves as the membrane source for both immature and mature autophagosomes in OA chondrocytes. This results in the creation of a reservoir that preemptively manages autophagy, which in turn reduces autophagy activity to handle further variations in ROS levels. As a result, this group that is susceptible to autophagy is why OA chondrocytes do not quickly decrease their autophagy response when faced with oxidative stress, which may even result in the death of chondrocytes due to autophagy.<sup>209,210</sup>

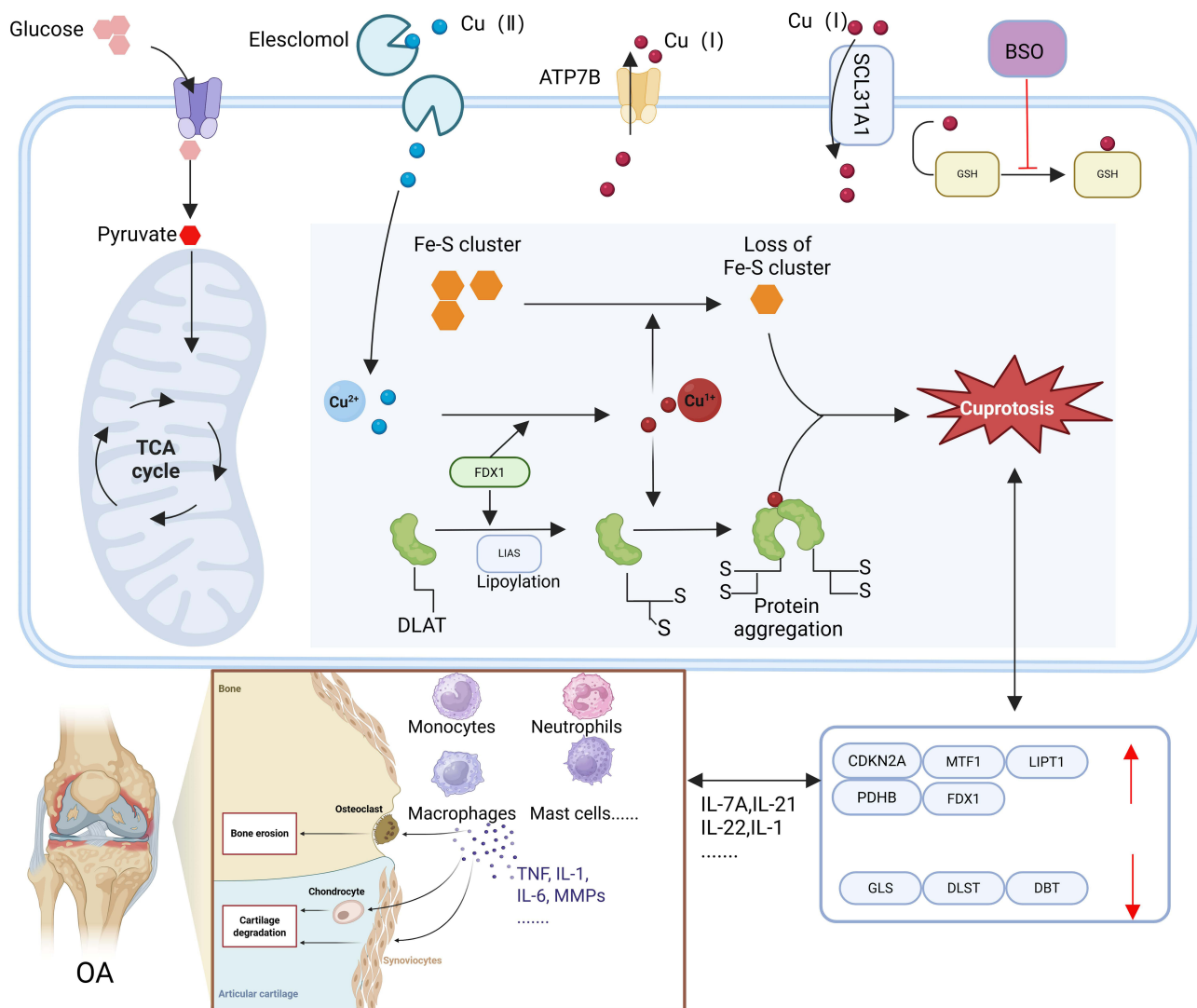
In summary, throughout the process of OA development, autophagy responds to several influences including aging, low oxygen levels, mechanical stress, inflammation, and oxidative stress. During this process, autophagy levels may rise as a defensive reaction to different pathological changes in the joint microenvironment.<sup>19,204,211</sup> However, if autophagy adaptation fails, it can result in the advancement of degenerative changes in the cartilage. However, even as we emphasize the protective function of autophagy, we must remain mindful of its dual nature. We propose that the bidirectional control of autophagy in relation to the survival or death of chondrocytes may be influenced by the specific type of cellular stress and the length of time the stress is applied.<sup>212</sup> Nonetheless, research on the impact of excessive autophagy on cartilage or chondrocyte function remains scarce. More investigation is required in the future to determine the thresholds that differentiate protective autophagy from excessive autophagy in chondrocytes across various pathological conditions. Consequently, we think that autophagy could serve as an important therapeutic target for articular cartilage in different stress scenarios. However, the therapeutic range and dosage of targeted autophagy medications still require additional investigation.

## Cuproptosis and Osteoarthritis

Cuproptosis is a unique type of cell death characterized by the buildup of copper in cells. This accumulation causes mitochondrial lipidation proteins to aggregate and destabilizes Fe-S cluster proteins, resulting in a form of cell death that is distinct from that induced by oxidative stress.<sup>213,214</sup>

## Disorders of Copper in Cartilage and Bone

About 66% of the copper within the human body is located in the muscles and skeletal structure. Numerous research have validated the significant function of copper in managing bone metabolism.<sup>215,216</sup> Copper has the ability to affect collagen production and bone matrix metabolism, thereby supporting healthy bone growth. Copper can improve bone mass and durability, in addition to encouraging bone growth.<sup>215,217,218</sup> Similarly, copper is essential for preserving the structure and balance of cartilage tissue. Under typical circumstances, copper (Cu) maintains a stable presence in cells, with its stability primarily dependent on three copper transporter proteins: SLC31A1 and ATP7A/B. SLC31A1 is involved in the absorption of copper, whereas ATP7A/B is involved in its elimination.<sup>219,220</sup> **Figure 4.** However, disruptions in copper balance, whether due to excessive accumulation or deficiency, can cause copper levels in cells to exceed or drop below the limits maintained by homeostatic processes.<sup>221</sup> This could result in harmful effects on bone and cartilage cells. Research has indicated that the level of copper in cartilage is greater than that found in other types of tissues within the knee joint, such as the meniscus and synovium.<sup>222</sup> Cu<sup>2+</sup> can enhance regeneration of cartilage and aid in the rebuilding of the junction between bone and cartilage, which helps reduce damage to cartilage tissue and speeds up the repair process.<sup>223</sup> Copper can influence the balance of cartilage by adjusting the function of hypoxia-inducible transcription factors and proteins associated with cartilage. Additionally, copper significantly boosts the chondrogenic differentiation of mesenchymal stem cells derived from bone marrow.<sup>224,225</sup> During the process of collagen production, copper functions as a cofactor of lysyl oxidase (LOX), which plays a key role in interlinking collagen in tissues that are abundant in cartilage. Furthermore, copper is involved in the metabolism of bone and cartilage by enhancing immune responses in cartilage and facilitating the development of articular cartilage and underlying bone.<sup>225,226</sup> Insufficient copper levels may interfere with the development of bones and cartilage, leading to potential harm to their structural



**Figure 4** The mechanism of cuproptosis and its impact on OA. DLAT: Dihydroceramide S-acyltransferase, FDX1: Ferroxidase 1, LIAS: Thiosulfate synthase, TCA: Tricarboxylic acid cycle, Fe-S cluster: Iron-sulfur cluster protein. Under ordinary conditions, copper maintains a stable level inside cells. The steady state of Cu predominantly relies on three copper transporters, namely ATP7A/BandSLC31A1. SLC31A1 is accountable for the uptake of copper into cells, whereas ATP7A/B is in charge of copper efflux. The Cu ion carrier ethylchelmonate is capable of transporting Cu<sup>2+</sup> into the cell, resulting in the accumulation of Cu within the cell. BSO restrains the chelating activity of the intracellular Cu chelator GSH. In the event of disruption of copper equilibrium, on the one hand, when there is an excessive accumulation of Cu<sup>2+</sup> in cells that are dependent on mitochondrial respiration, Cu<sup>2+</sup> combines with sulfenic acid DLAT, resulting in abnormal oligomerization of DLAT. The augmentation of insoluble DLAT gives rise to cytotoxicity and triggers cell death; On the other hand, FDX1 converts Cu<sup>2+</sup> into a more toxic form of Cu<sup>+</sup>, this will result in the suppression of Fe-S cluster protein synthesis and trigger cell death. Furthermore, CRGs are associated with cellular factors influencing synovial cells, it has an impact on diverse cell types in OA, like neutrophils, monocytes, and macrophages. Created in BioRender. Jiwei, H (2025) <https://BioRender.com/n41i521>.

integrity. Copper deficiency could impact the reactions facilitated by different enzymes that require copper functioning as a cofactor. A significant result is the deterioration of LOX, which hinders the linking of peptide chains.<sup>227</sup> Consequently, the matrix of bone is weakened, the cartilage becomes more delicate, and the body’s vulnerability to cartilage breaks increases. In the end, the cartilage’s integrity is weakened.<sup>228</sup> This damage may be associated with HIF activation that is mediated by copper. An interruption in the HIF-1 $\alpha$  signaling pathway can affect the production of energy and the synthesis of biological molecules in cells, potentially resulting in problems related to skeletal development.<sup>226,229,230</sup> However, elevated copper levels can hinder the process of rat bone marrow mesenchymal stem cells (MSCs) differentiating into osteoblasts. It is widely thought that this phenomenon happens due to the stabilization of the HIF-1 $\alpha$  protein and the reduced expression of a gene known as Runx2, both of which play crucial roles in normal bone development.<sup>217</sup> Copper can directly prevent the breakdown of bone carried out by osteoclasts.<sup>231</sup> The experiment

showed a notable reduction in the number of identified mature osteoclasts when the  $\text{Cu}^{2+}$  concentration reached 20  $\mu\text{m}$  or more. As the levels of  $\text{Cu}^{2+}$  increase, there is a notable reduction in the number of osteoclasts.<sup>232</sup> Earlier research has indicated a link to mitochondrial processes, copper proteins are capable of effectively removing ROS from both external and internal sources, thus helping to restore mitochondrial function and manage chondrocyte metabolism.<sup>233</sup> Nevertheless, differing opinions continue to exist, and a definitive conclusion has not been achieved. A lack of copper can decrease the function of monoamine oxidase and Lox, which can result in the production of reactive ROS by cells. As a result, the redox balance in osteoblasts becomes disrupted, which reduces their survival capacity and hinders their development.<sup>234,235</sup> Concurrently, the solubility of collagen rises, the peptide chains break down, and the collagen loses its stability, which ultimately results in reduced bone strength.<sup>236,237</sup> In tissue engineering, particularly regarding regeneration of cartilage, the addition of metal ions can improve cell adhesion, improve cell performance, and enhance the potential for regenerating cartilage tissue.<sup>238,239</sup> Currently, the research on copper ions is facing some limitations. The research into whether raising copper concentrations in injured cartilage, which has low copper content, can promote the healing of cartilage tissue is a topic that merits additional investigation, especially regarding copper's role.<sup>240</sup> Too much copper may harm cartilage, but the damage can be effectively repaired based on the severity of the cartilage injury. In the initial phases of WD, research indicated the presence of copper deposits in the cartilage and synovial tissue of patients with OA.<sup>241,242</sup> Li<sup>243</sup> et al also noted that copper is found in cartilage that is significantly damaged, as opposed to cartilage that shows only mild wear. This discovery indicates that an increase in copper levels is associated with the exacerbation of cartilage damage.<sup>243</sup> Crucially, adding copper to chondrocyte cultures could result in changes to the cell's cytoskeleton, such as differences in microfilaments, microtubules, and the arrangement of fibronectin.<sup>244</sup> The deactivation of integrin-associated kinase in chondrocytes could lead to a decrease in levels of cyclin D1, which could hinder the proliferation of chondrocytes.<sup>245</sup> Nonetheless, the exact ways in which copper accumulation impacts cartilage require further investigation. Conducting thorough research on how cell therapy affects copper levels, both in the body and in connection with relevant scaffolds, alternative materials, and other groundbreaking innovations, is anticipated to provide valuable insights for future research initiatives.

### Cuproptosis in Bone and Cartilage Biology

In March 2022, Tsvetkov et al were the first to suggest that copper ions could lead to cell death.<sup>246</sup> They found a distinct type of cell death triggered by copper, which is different from the three types of cell death mentioned above. They named the recently identified copper-dependent cell death mechanism "cuproptosis." The primary way copper causes cell death is through the buildup of copper ions inside the cell.<sup>246</sup> Copper is transported into the cell through the  $\text{Cu}^{2+}$  carrier elesclomol, while the reduction in endogenous Cu chelator GSH in the cell leads to an increase in intracellular  $\text{Cu}^{2+}$  levels.<sup>247</sup> A higher concentration of intracellular  $\text{Cu}^{2+}$  could influence the association of  $\alpha$ -lipoic acid within the tricarboxylic acid cycle, potentially interrupting the cycle and resulting in protein clumping and imbalance.<sup>214</sup> Copper ions additionally reduce the levels of iron-sulfur (Fe-S) cluster proteins, leading to stress caused by protein toxicity.<sup>214</sup> The process of DLAT oligomerization and the decrease in Fe-S stability ultimately result in cell death<sup>246</sup> Figure 4. In the past few years, the importance of copper in the biology of bone and cartilage has been increasingly uncovered. CRGs are the genes associated with cell death triggered by copper ions, including GLS, MTF1, DLAT, CDKN2A, PDHA1, PDHB, as well as DLD. Research has demonstrated that these genes affect the biology of bone and cartilage by regulating the functions of chondrocytes, osteoclasts, and osteoblasts.<sup>248</sup> DLAT is abundantly expressed in chondrocytes, resulting in their death and subsequent cartilage damage. At the same time, blocking DLAT could lead to a reduction in chondrocyte death.<sup>249</sup> MTF1 is significantly expressed in chondrocytes and FLS, which contributes to the development of osteophytes, subchondral bone sclerosis, cartilage damage, and inflammation of the synovial membrane. On the other hand, the lack of the MTF1 gene can successfully prevent the breakdown of cartilage and subchondral bone hardening in mice.<sup>250</sup> GLS can influence the differentiation of kidney cells by modulating histone acetylation.<sup>251</sup> The regulation of cell fate and bone formation is influenced by GLS and glutamine metabolism during the differentiation process. Furthermore, the osteogenic and adipogenic differentiation of SSCs can also be affected by GLS and glutamine metabolism.<sup>252</sup> The protein of P16 (INK4A), which is produced by the CDKN2A gene, is a common indicator of cellular aging. An overproduction of this protein could hinder the mineralization process in osteoblasts and lead to a rise in the quantity of osteoclasts.<sup>253</sup> Earlier research has also outlined the influence of CRGs on the formation of osteoclasts and osteoblasts.

Inhibiting PDHA1 could encourage the growth of osteoblasts, whereas activating PDHA1 may influence both macrophages and osteoblasts by reducing the secretion of inflammatory substances.<sup>220</sup> PDHB could impact the differentiation of osteoclasts by interacting with kinase 10 associated with nima, or it may also influence the differentiation of osteoblasts by affecting mitochondrial balance.<sup>254</sup> DLD influences glycolysis, which in turn impacts the differentiation of osteoclasts.<sup>254</sup> In synovitis tissue, the CRGs that are expressed at high levels include PDHA, LIPT1, FDX1, PDHB, and CDKN2A, whereas DBT and DLST are expressed at lower levels. It is believed that certain CRGs may influence the normal physiological functions of the synovium and modulate the activity of synovial cells<sup>255,256</sup> Figure 4. At present, the effects of CRGs on the biology of bone and cartilage remains uncertain, and the possible mechanisms behind CRGs are not well understood. However, earlier research has indicated that many genes influence bone formation and resorption, either directly or indirectly. In the forthcoming future, these associated genes have significant potential for progress in bone and cartilage biology. Therefore, additional study is needed to investigate them further.

## The Mechanisms of Chondrocyte Apoptosis in Osteoarthritis

Apoptosis is an active cell death process regulated by intrinsic genetic mechanisms under specific physiological or pathological conditions. It is also known as programmed cell death (PCD) or type I cell death.<sup>24</sup> Morphologically, it is characterized by cell shrinkage, loss of connections, and separation from surrounding cells, accompanied by cytoplasmic condensation, disappearance of mitochondrial membrane potential, nuclear mass aggregation, nucleolar fragmentation, and degradation of DNA into 180–200 bp fragments. Finally, apoptotic vesicles with intact cytoplasmic structures are formed without triggering an inflammatory response.<sup>257,258</sup> Apoptosis can be categorized into exogenous (death receptor pathway) and endogenous (mitochondrial pathway) types.<sup>258</sup> In the exogenous pathway, death receptors (such as Fas, TNFR1, TRAILR, members of the TNFRSF family)<sup>259</sup> bind to death ligands, causing conformational changes in the cell membrane and activating caspases, ultimately leading to cell apoptosis.<sup>257</sup> In osteoarthritis (OA), apoptosis plays a crucial role in the deterioration of articular cartilage. Exogenously, synovial inflammation is common in OA patients, and inflammatory mediators (such as ROS, IL, NO, matrix-degrading enzymes, etc) promote chondrocyte death through specific pathways.<sup>135</sup> For instance, the death domain of TNFR-1 binds to the FADD protein, recruits and activates Caspase-3, 7, and 8, forms a death-inducing signaling complex, and triggers chondrocyte apoptosis.<sup>260,261</sup> Certain drugs (such as oxflor and sparflor) can also promote chondrocyte apoptosis by activating the TNFR signaling pathway. Endogenously, factors like DNA damage, endoplasmic reticulum stress, hypoxia, high cytoplasmic Ca<sup>2+</sup> concentration, and severe oxidative stress can trigger the mitochondrial apoptosis pathway.<sup>258</sup> This causes the opening of the mitochondrial permeability transition pore, release of cytochrome c, Smac, AIF, etc., and activation of the caspase cascade, resulting in chondrocyte apoptosis. For example, an increase in intracellular Ca<sup>2+</sup> activates calpain, which cleaves membrane-bound AIF, and the soluble AIF fragments are released outside the mitochondria to induce apoptosis.<sup>258</sup> In addition, inflammatory mediators such as ROS and inducible nitric oxide synthase,<sup>262</sup> as well as IL-1 $\beta$  (which can damage mitochondrial DNA, interfere with chondrocyte metabolism, enhance TNF- $\alpha$  activity, etc),<sup>263</sup> are closely related to chondrocyte apoptosis in OA. High levels of ROS can also damage cell membranes, nucleic acids, and mitochondria, exacerbating inflammation and cell death.<sup>264</sup> Currently, the molecular regulatory mechanisms of signaling pathways related to apoptosis in OA and the correlations among various factors remain incompletely understood. Future research on targeted drugs based on these findings is expected to provide new insights for the early prevention and treatment of OA.<sup>263–266</sup>

## Results Programmed Cell Death Drugs in Clinical Application for Osteoarthritis

### Targeted Therapy for Ferroptosis in Osteoarthritis

The elements of traditional Chinese medicine are significant in the regulation of ferroptosis. *Icariin* (ICA) has the ability to alleviate the decrease in GPX4, SLC7A11, and SLC3A2L levels in synovial cells affected by OA due to LPS, and it also reduces the levels of malondialdehyde (MDA) and iron. Studies indicate that ICA is capable of preventing ferroptosis in synovial cells induced by LPS affected by osteoarthritis by downregulating the iron transport protein TFR1 and enhancing the system Xc/GPX4 regulatory pathway.<sup>267</sup> *Myristicin* (CAD), a compound derived from ginger

plants, has been shown to lower levels of iron, ROS, and MDA in chondrocytes that have deteriorated due to IL-1 $\beta$  exposure. Additionally, it enhances the expression of the proteins GPX4 and SLC7A11. This indicates that CAD could greatly reduce ferroptosis in chondrocytes that is triggered by IL-1 $\beta$ .<sup>268</sup> The proposed mechanism could activate the GPX4/SLC7A11 signaling pathway, which might help decrease ferroptosis in cartilage cells.<sup>268</sup> *Biochanin A* (BCA) is an active ingredient found in *Astragalus*. In a study involving iron overload in chondrocytes derived from mice treated with FAC, BCA has the ability to counteract the increased levels of the iron transport protein TFR1 and the decreased levels of FPN, thus helping to manage the labile iron pool (LIP) in chondrocytes.<sup>98</sup> It may also boost the expression levels of the Xc-system, GSH, and GPX4 proteins, which would strengthen the protective processes against lipid peroxidation and decrease the buildup of ROS caused by excess iron. Research indicates that BCA might reduce cellular iron levels by blocking TFR1 and enhancing the expression of FPN.<sup>98</sup> Additionally, controlling the Xc-system/GPX4 pathway is crucial for regulating the buildup of ROS, which helps prevent lipid peroxidation and slows down the advancement of OA.<sup>98</sup> Research indicates that *Baicalin*, a substance derived from *astragalus* extract, can lower the levels of MMP13, ADAMTS5, and Col1 that are triggered by IL-1 $\beta$  in chondrocytes impacted by osteoarthritis.<sup>269</sup> Additionally, it enhances the expression of Col2 while reducing the amounts of lipid ROS and Fe<sup>2+</sup>. The findings indicate that *Baicalein* can prevent the breakdown of the ECM caused by IL-1 $\beta$ . Additionally, it encourages ECM production in human chondrocytes impacted by OA by inhibiting ferroptosis, which may help slow down the progression of OA.<sup>269</sup> The polyphenolic compound *Theaflavin-3,3'-digallate* (TF3), found in black tea, has demonstrated the ability to counteract the decrease of FTH1, GPX4, and SLC7A11 caused by erastin in chondrocytes from individuals with OA.<sup>270</sup> Furthermore, it lowers the levels of ROS in cells and enhances Fe<sup>2+</sup> storage through FTH1, thereby aiding in the reduction of ferroptosis associated with OA.<sup>270</sup> The findings indicate that focusing on important molecules associated with ferroptosis, including ROS, GPX4/SLC7A11, GSH, FTH1, TFR1, FPN, and Fe<sup>2+</sup>, through the active ingredients of traditional Chinese medicine could provide a hopeful approach for treating OA in the future. (Table 1) Research shows that semaphorin 5A prevents ferroptosis, upregulates GPX4, and modulates the SREBP1/SCD-1 pathway via the PI3K/AKT/mTOR axis in rheumatoid arthritis. Given the shared synovial inflammation and tissue degradation in OA, semaphorin 5A likely plays a similar regulatory role in OA, making it a potential target for both conditions.<sup>271</sup> Metformin has demonstrated promise in the

**Table 1** Potential Drugs That Can Inhibit Ferroptosis in OA

| Drugs                                | Molecular Mechanism   | Effect   |
|--------------------------------------|---|--|
| Icariin (ICA)                        | The expression of TFR1 is inhibited; The systemXc-/GPX4 regulatory axis is excited at the same time   | Ferroptosis of synovial cells is inhibited in OA   |
| Myristicin (CAD)<br>BiochaninA (BCA) | The P53/SLC7A11/GPX4 axis is activated<br>Iron levels and the Nrf2/SystemXc-/GPX4 axis are regulated  | Ferroptosis of chondrocytes is inhibited in OA<br>Eliminate ROS accumulation and prevent lipid peroxidation, thus delaying the progression of OA |
| Baicalein                            | The expression of MMP13, ADAMTS5 and collagen I (Col1) in OA chondrocytes is decreased; the expression of Col2 is increased; the contents of ROS and Fe <sup>2+</sup> are decreased   | Increase the anabolism of ECM in human OA chondrocytes, thereby delaying the progression of OA   |
| Theaflavin-3,3'-digallate (TF3)      | The down-regulated expression of FTH1, GPX4 and SLC7A11 in OA chondrocytes is reversed  | ROS is reduced in OA chondrocytes and Fe <sup>2+</sup> storage of FTH1 is promoted   |
| Deferoxamine (DFO)                   | The Nrf2/GPX4 signaling pathway is activated  | The Fe <sup>2+</sup> content of chondrocytes is decreased in OA  |
| Ferrostatin-I (Fer-I)                | Oxygen free radicals are removed; The increase of ROS, MDA and Fe <sup>2+</sup> contents in OA chondrocytes is reversed; The decrease of GPX4, GSH and GSH/GSSG protein expression is reversed; The decrease in GPX4 and FTH1 mRNA levels is reversed | Chondrocyte ferroptosis is inhibited in OA   |
| Semaphorin 5A                        | Up-regulated GPX4, PI3K/AKT/mTOR signaling pathway and SREBP1/SCD-1 signaling axis  | Ferroptosis of synovial fibroblasts is inhibited in OA   |

(Continued)

**Table 1** (Continued).

| Drugs        | Molecular Mechanism  | Effect  |
|--------------|--|---|
| Metformin    | Up-regulated the GPX4 regulatory axis  | Chondrocyte death is inhibited in OA; The decrease in subchondral bone sclerosis and angiogenesis is reversed         |
| D-mannose    | The susceptibility of chondrocytes to ferroptosis is suppressed  | Ferroptosis of chondrocytes is inhibited  |
| Stigmasterol | Ferroptosis is inhibited; Cell viability is increased by negative regulation of sterol regulatory element binding transcription factor 2 (SREBTF2) | Proinflammatory factors in chondrocytes are suppressed; Degradation of the ECM is inhibited; Ferroptosis is inhibited |

treatment of OA by enhancing the expression of Gpx4, which helps safeguard cartilage cells from dying. This process not only relieves OA symptoms but also contributes to some improvement in subchondral bone sclerosis and decreases angiogenesis in mouse models.<sup>272</sup> Research studies have demonstrated that sitosterol derived from *Achyranthes bidentata* can inhibit pro-inflammatory factors in chondrocytes and reduce the breakdown of the ECM.<sup>273</sup> Cheng<sup>274</sup> and his team discovered that stigmasterol can prevent ferroptosis triggered by IL-1 $\beta$ , thereby improving cell survival. This process works by inhibiting the interaction between sterol regulatory element-binding transcription factor 2 (SREBTF2) and the sterol regulatory element.<sup>274</sup> D-Mannose is a natural substance present in various plants and their excretions, which has demonstrated the ability to reduce the vulnerability of chondrocytes to cell death, particularly in relation to ferroptosis induced by HIF-2 $\alpha$ . This effect could potentially help relieve symptoms related to OA.<sup>274</sup> Iron chelators are compounds that attach to iron, helping to remove it from the body. These medications function by inhibiting the Fenton reaction and providing antioxidant benefits in the body.<sup>275</sup> Desferrioxamine (DFO) is a potent iron-binding agent utilized in the treatment of conditions associated with the buildup of excess iron in the body.<sup>276,277</sup> The findings indicate that DFO may help to slow the advancement of OA, probably by stimulating the Nrf2 pathway. This stimulation could play a role in lowering Fe<sup>2+</sup> levels in OA chondrocytes and preventing ferroptosis associated with OA.<sup>94</sup> Fer1 acts as a fat-soluble antioxidant and specifically prevents ferroptosis. It aids in eliminating oxygen free radicals generated by iron in lipid hydroperoxides, It has the ability to generate an effect that counteracts ferroptosis, akin to that of GPX4.<sup>278</sup> Research has indicated that Fer1 reduced the elevated levels of ROS, malondialdehyde (MDA), and Fe<sup>2+</sup>, while also counteracting the reduced expression of GPX4, GSH, and the GSH/GSSG ratio caused by TBHP in chondrocytes impacted by OA.<sup>279</sup> Additionally, it restored the mRNA expression levels of GPX4 and FTH1 that had been lowered. The findings suggest that Fer1, recognized for its function as a blocker of iron-related cell death, is capable of stopping TBHP-triggered ferroptosis in chondrocytes affected by OA.<sup>90</sup> It does this by decreasing lipid peroxidation, regulating iron buildup, and preserving the activity of GPX4/GSH.<sup>90</sup> In conclusion, focusing on specific molecules related to iron overload and lipid peroxidation could be a significant strategy for preventing and treating OA (Table 2).

## Targeted Therapy for Cell Pyroptosis in Osteoarthritis

Since pyroptosis is strongly linked to inflammation, it is important to take both factors into account when creating a treatment plan for OA.<sup>280</sup> *Matrine* and *Morroniside* can decrease chondrocyte pyroptosis and the inflammatory response by blocking the activation of NF- $\kappa$ B.<sup>281,282</sup> The research indicated that a diet high in unsaturated fatty acids may have anti-inflammatory and anti-pyroptotic properties by blocking the TLR4/NF- $\kappa$ B signaling pathway as well as the NLRP3, caspase-1 or GSDMD pathway, which could help lessen the impact of OA.<sup>283</sup> In the OA rat model, metformin, a medication often used to treat diabetes, lowers NLRP3 levels and helps reduce pyroptosis in cartilage cells due to its inhibitory properties.<sup>284</sup> Exosomes obtained from mesenchymal stem cells, along with microRNA, phytochemicals, growth factors, and biological enzymes, have been shown to decrease chondrocyte pyroptosis, indicating their promising ability to inhibit this process. Furthermore, blocking pathways associated with pyroptosis can help relieve OA by decreasing pyroptosis<sup>124,285–297</sup> (Table 2). Inflammatory bodies play a crucial role in starting and regulating pyroptosis by detecting different DAMPs and PAMPs, which activates pro-inflammatory caspases. Therefore, inhibiting the

**Table 2** Strategies to Inhibit Chondrocyte Pyroptosis, Including Phytochemicals, Exosomes, microRNAs, Growth Factors, Biological, Enzymes, and Physical Therapy

| Name  | Pathway(s)  | Function(s)   |
|---|---|---|
| Icariin   | The expression of NLRP3 inflammasome is decreased   | The pyroptosis of chondrocytes is inhibited; The production of inflammatory factors is reduced;ECM degradation is reduced                             |
| Quercetin   | The expression of NLRP3 inflammasome is decreased   | The pyroptosis of chondrocytes is inhibited; Inhibit apoptosis; Oxidative stress is suppressed; ECM degradation is inhibited                          |
| Bone marrow MSC-derived exosomes                              | Dac3-mediated STAT1 and NF-κB pathways are inhibited  | The pyroptosis of chondrocytes is inhibited;The production of inflammatory factors is reduced; ECM degradation is reduced                             |
| Ubiquitin-specific protease7 Inhibition (USP7 Inhibition)     | NOX4/ROS/NLPR3 is inhibited   | Chondrocyte pyroptosis is inhibited; Oxidative stress is suppressed; The production of inflammatory factors is reduced; ECM degradation is reduced    |
| Adenosine A3 receptor   | NLRP3/caspase-1/ GSDMD-induced signaling is inhibited   | The pyroptosis of chondrocytes is inhibited in OA; Anti-inflammatory; Pain is alleviated; ECM degradation is inhibited; The progress of OA is delayed |
| Exogenous stromal cell-derived factor-1 (SDF-1) P2X7 receptor | Activation of AMPK signaling pathway inhibits NLRP3 inflammasome  | Synovial cell inflammation and pyroptosis are inhibited in OA   |
| miR-140-5p  | CTSB/NLRP3 is inhibited   | The pyroptosis of chondrocytes is inhibited in OA; The cartilage damage is alleviated   |
| miR-155 knockdown   | The NLRP3/Caspase-1 pathway is inhibited by targeting SMAD2   | The pyroptosis of chondrocytes is inhibited in OA; The viability of chondrocytes is enhanced  |
| miR-107   | The expressions of caspase-1, c-caspase-1, GSDMD-N and TLR4 are down-regulated in chondrocytes  | The pyroptosis of chondrocytes is inhibited in OA; It promotes the secretion of collagen by chondrocytes  |
| Moderate-intensity exercise                                   | The P2X7/AMPK/mTOR signaling axis is activated  | The autophagy of chondrocytes is promoted in OA;The pyroptosis of chondrocytes was inhibited  |
| Moderate-intensity exercise-induced irisin                    | The PI3K/Akt/NF-κB signaling pathway is inhibited   | The pyroptosis of chondrocytes is inhibited; Anti-inflammatory  |
| Electro-acupuncture   | The expression levels of NLRP3, ASC, Caspase-1, IL-1β, IL-18 mRNA and protein, as well as GSDMD mRNA and GSDMD-N protein in synovium are down-regulated | Reduces inflammation of synovial tissue in OA   |
| Loganin   | Activation of the NF-κB pathway is blocked  | Degeneration of cartilage, synovitis and joint pain are suppressed  |
| Ursolic acid  | Activation of the NF-κB pathway is blocked  | Degeneration of cartilage, synovitis and joint pain are suppressed  |
| Chondroitin sulphate  | Activation of the NF-κB pathway is blocked  | Degeneration of cartilage, synovitis and joint pain are suppressed  |
| KMU-1170  | Phosphorylation is inhibited in the NF-κB pathway; mRNA transcription of NLRP3 inflammasome is inhibited  | Inflammation in the FLS is suppressed   |
| Amitriptyline   | Bind to TLR4 and block NF-κB signaling pathway  | Chondrocyte degradation, synovial inflammation and osteoblast destruction are inhibited in OA   |
| Loratadine  | Inhibition of the NF-κB pathway by the Nrf2/HO-1 axis   | Inflammation, oxidative stress, and degradation of chondrocytes are inhibited in OA   |
| Lico A  | The expression of NLRP3 gene was inhibited  | Inflammation, oxidative stress, and degradation of chondrocytes are inhibited in OA   |
| CY-09   | The expression of NF-κB/ NLRP3 is inhibited   | Pyroptosis, catabolism and inflammatory responses of chondrocytes are inhibited   |
| Chrysin   | The expression of NF-κB/ NLRP3 is inhibited   | Inflammation of the synovium and OA pain are suppressed   |

(Continued)

**Table 2** (Continued).

| Name                    | Pathway(s)   | Function(s)   |
|-------------------------|--|---|
| Baicalein               | The expression of NF- $\kappa$ B/ NLRP3 is inhibited | Pyroptosis, catabolism and inflammatory responses of chondrocytes are inhibited |
| Less mechanical loading | The expression of NF- $\kappa$ B/ NLRP3 is inhibited | Pyroptosis, catabolism and inflammatory responses of chondrocytes are inhibited |

activation and formation of inflammasomes could safeguard cells from undergoing pyroptosis and the related issues that come with it. At present, scientists are concentrating on the NLRP3 inflammasome and the signaling pathways that control it, as it can react to different stimuli that lead to cellular disruptions.<sup>298</sup> As previously stated, the NF- $\kappa$ B pathway is essential for the triggering of the NLRP3 inflammasome. Therefore, blocking the activation or transmission of the NF- $\kappa$ B pathway is the preferred method for targeted inhibition of pyroptosis.<sup>299</sup> Creating a regulatory pathway involving NF- $\kappa$ B/NLRP3 inflammation using *Marasmius*, *Mrsolic acid*, and chondroitin sulfate to reduce cartilage deterioration and alleviate synovitis and OA pain in rats.<sup>300–302</sup> In 2021, Baek<sup>303</sup> developed a novel multi-protein kinase inhibitor known as KMU-1170, which is derived from indole-2-one. They subsequently found that it could successfully inhibit nearly all phosphorylation activities in the NF- $\kappa$ B signaling pathway, leading to a decrease during the mRNA transcription process of the NLRP3 inflammasome.<sup>303</sup> Additionally, the Franco-Trepatt team found that the antidepressant amitriptyline has the ability to bind to TLR4, effectively inhibiting the NF- $\kappa$ B signaling pathway. This action prevents the activation of NLRP3 in OA chondrocytes, synovial cells, and osteoblasts, ultimately impeding the secondary immune response.<sup>304</sup> Furthermore, the NLRP3 inflammasome needs a secondary signal for its activation and assembly following the receipt of the primary activation signal. Research has indicated that ROS are essential for the activation and formation of the NLRP3 inflammasome.<sup>305</sup> As a result, enhancing the state of oxidative stress within cells has emerged as another effective approach. The antihistamine loratadine's component licochalcone A (Lico A) and licorice extracts serve as regulatory agents for Nrf2, helping to reduce the NLRP3 inflammasome in response to ROS stimulation. The first mechanism reduces the NF- $\kappa$ B pathway through the Nrf2/HO-1 axis, while the second enables Nrf2 to enter the cell nucleus and directly inhibit the triggering of the NLRP3 gene.<sup>306,307</sup> Numerous pharmacological treatments, including the NLRP3 inhibitor CY-09,<sup>124</sup> *Curcumin*,<sup>308</sup> and *Baicalin* (delivered via intra-articular injection),<sup>120</sup> along with physical therapy methods like decreasing mechanical stress on the knee joint<sup>119</sup> and applying electrical stimulation,<sup>309</sup> have demonstrated effectiveness in lowering NLRP3 inflammasome expression. These approaches help prevent chondrocyte pyroptosis, metabolic degradation, and inflammatory reactions, ultimately leading to improved cartilage health. Nonetheless, these research works failed to investigate the molecular mechanisms within animal models, indicating that future research should focus on further exploring and advancing this area. GSDMD experiences pyroptosis as it processes a wide array of both internal and external signals from the inflammasome.<sup>310,311</sup> If the GSDMD pore does not form properly, it will impede the function of most previously activated inflammasomes, along with their related cysteine proteases and biochemical signals. By inhibiting the widespread activation of GSDMD, which initiates pyroptosis and subsequently diminishes the inflammatory response, this approach aids in preventing pyroptosis. As a result, inhibiting GSDMD demonstrates greater selectivity than using particular inflammasome inhibitors or treatments aimed at IL-1 $\beta$  and IL-18. Consequently, from a theoretical standpoint, focusing on the inhibition of GSDMD could be the most promising approach for future treatments. Currently, strategies aimed at specifically inhibiting GSDMD can be categorized into two main approaches: the first approach focuses on decreasing the cleavage of GSDMD by inflammatory cysteine proteases, while the second approach targets the prevention of oligomerization and/or membrane insertion of GSDMD-nto.<sup>309</sup> Yang<sup>312</sup> developed a GSDMD-derived inhibitor called N-Acetyl-phenylalanine-leucine-threonine-aspartic acid-chloromethyl ketone (Ac-FLTD-CMK) by pinpointing the cleavage site at which GSDMD interacts with caspase. They discovered that it substituted for full-length GSDMD and interacted with the previously mentioned caspase, inhibiting cleavage.<sup>312</sup> Furthermore, experiments involving covalent modification of human GSDMD cyc191 and

mouse GSDMD cyc192 revealed that disulfiram and necrosulfonamide, which are inhibitors of pyroptosis, were unable to trigger the oligomerization of GSDMD-NT, It can successfully prevent the formation of pores both in living organisms and in laboratory settings.<sup>313,314</sup> Our research indicates that the chemical compound LDC7559 exhibits a level of effectiveness comparable to other medications aimed at GSDMD, likely because it interacts with GSDMD-NT.<sup>315</sup> Additionally, cell biology researchers have explored the potential for cells to mend GSDMD pores in their membranes by forming vesicles that can enclose and later release these pores into the surrounding environment.<sup>316</sup> Furthermore, another study investigated the function of GSDMD in post-traumatic OA. Eliminating GSDMD reduces cartilage alterations, synovitis, and subchondral bone hardening.<sup>317</sup> In conclusion, GSDMD represents a significant advancement in the treatment and management of conditions associated with pyroptosis. Nevertheless, because there is insufficient detailed research on the pyroptotic molecular mechanisms in cell types beyond immune cells, and given that inhibitors targeting GSDMD do not influence the initial inflammatory processes associated with pyroptosis, further experiments are required to clarify the precise molecular mechanisms and effects of GSDMD-targeted inhibitors across various diseases.

## Targeted Treatment of Mitochondrial Autophagy in Osteoarthritis

A thorough and detailed knowledge of the targets associated with mitochondrial autophagy and targeted therapies is crucial for the treatment of OA. Focusing on mitochondrial autophagy and swiftly removing damaged mitochondria is crucial for maintaining the normal physiological function of chondrocytes.<sup>175,318</sup> This strategy can effectively prevent and reduce cartilage degeneration associated with OA. The primary therapeutic agents aimed at modulating mitochondrial autophagy in OA consist of active ingredients from conventional Chinese medicine, dissolvable amino acids, and beneficial bacteria generated through gut microbiota metabolism, the trace element zinc, metformin, and exosomes.<sup>83,319,320</sup> The elements of traditional Chinese medicine are significant in the regulation of mitosis. *Baicalin*, a natural substance extracted from the desiccated root of *Scutellaria baicalensis*, has demonstrated properties that reduce inflammation, combat tumors, and modulate the immune system.<sup>321</sup> *Baicalin* enhances the level of Bcl-1, Beclin3, LC3-II/LC3-I, p-Drp1, PINK1, and Parkin in chondrocytes of rats stimulated by IL-1 $\beta$ . This action supports cell survival, autophagy, and mitophagy in these chondrocytes, while also elevating MMP levels.<sup>322</sup> It has been noted that *Baicalin* can prevent the induction of apoptosis in rat chondrocytes by IL-1 $\beta$ . The findings indicate that *Baicalin* could stimulate IL-1 $\beta$ -induced mitophagy via the PINK1/Parkin pathway, which may help reduce damage to chondrocytes.<sup>322</sup> *Curcumin*, a polyphenolic compound derived from turmeric rhizomes, has the potential to greatly improve the gene and protein expression of mitotic markers such as PINK1, Parkin, LC3B, P62, and Beclin1 in chondrocyte degeneration caused by IL-1 $\beta$ .<sup>323</sup> It can also suppress the expression of the genes and proteins associated with the markers of inflammation IL-1 $\beta$  and MMP13. This suggests that *Curcumin* (turmeric extract) may slow down the deterioration of cartilage by stimulating mitochondrial autophagy in chondrocytes.<sup>323</sup> These research findings indicate that the mitochondrial autophagy influenced by the active ingredients in traditional Chinese medicine could be a promising area for future OA therapies. (Table 3) Studies have shown that *Irisin* constitutes a soluble peptide made up of 112 amino acids, playing a role in the regulation of bone remodeling and the maintenance of muscle balance.<sup>324</sup> *Irisin* has the ability to boost the levels of PINK1 and Parkin in chondrocytes of mice stimulated by IL-1 $\beta$ , promote mitochondrial autophagy, decrease excessive mitochondrial fission, enhance mitochondrial fusion, and slow down the advancement of OA.<sup>325</sup> This indicates that the recombinant *Irisin* protein can enhance mitochondrial autophagy triggered by IL-1 $\beta$  in mouse chondrocytes, manage mitochondrial dynamics, and ultimately help prevent the advancement of OA.<sup>325</sup> Research indicates that zinc provides a protective benefit to SW1353 human chondrosarcoma cells that have been exposed to methyl iodide acetate (MIA).<sup>326</sup> Zinc has the ability to mitigate cartilage damage resulting from MIA treatment by restoring low ATP levels and enhancing mitochondrial autophagy pathways associated with Parkinson's disease. This suggests that zinc may offer protective benefits against OA.<sup>326</sup> Research has shown that metabolites from the gut microbiome, including uric acid (UA), can stimulate the PINK1/Parkin pathway in patients with OA.<sup>327</sup> This activation helps to prevent the degeneration of cartilage cells and enhances mitochondrial autophagy, leading to potential therapeutic benefits.<sup>327</sup> Additionally, certain research has indicated that metformin can enhance mitochondrial autophagy by increasing levels of SIRT3, PINK1, and Parkin, along with the LC3II/LC3I ratio, in OA degenerative chondrocytes activated by IL-1 $\beta$  in mice. Metformin can reduce the expression of MMP13 and MMP3 while increasing the expression of Col2, which helps to mitigate the ECM

**Table 3** Potential Drugs That Can Regulate Mitophagy in OA

| Drugs           | Molecular Mechanism  | Effect   |
|-----------------|--|--|
| Baicalin        | The PI3K/AKT/mTOR pathway is inhibited; The PINK3/Parkin and PINK1/Drp-1 pathways are activated  | It promotes cell viability, autophagy and mitophagy of chondrocytes, increases MMP, and reduces chondrocyte injury             |
| Curcumin        | The AMPK/PINK1/Parkin pathway is activated   | The gene and protein expression of MMP13 and IL-1 $\beta$ are inhibited; Mitophagy is enhanced to delay cartilage degeneration |
| Irisin          | The expressions of PINK1, Park, PGC-1 $\alpha$ and TFAM are increased  | Enhancing mitochondrial autophagy of chondrocytes, regulate mitochondrial dynamics, and delay the progression of OA            |
| Zinc            | Upregulation of PINK1/Parkin-dependent mitochondrial autophagy   | Mitophagy is enhanced to protect OA chondrocytes from injury   |
| UrolithinA (UA) | Pink1-parkin-mediated mitochondrial autophagy is activated   | Impaired chondrocyte mitophagy in OA is enhanced to reduce the degeneration of OA chondrocytes                                 |
| Metformin       | The SIRT3/PINK1/Parkin signaling pathway is enhanced; The expression of MMP3 and MMP13 is down-regulated; The expression of Col2 is up-regulated | Mitophagy is promoted in damaged chondrocytes in OA; ECM catabolism is inhibited in degenerative chondrocytes                  |
| BMSC-exosomes   | LC3-II/ LC3-I and Beclin-1 protein expression are increased  | Chondrocyte mitophagy is enhanced in OA to inhibit chondrocyte apoptosis   |

degradation caused by IL-1 $\beta$  in OA degenerative cartilage cells in mice.<sup>328</sup> However, the SIRT3 inhibitor 3-TYP can counteract these effects, indicating that SIRT3 may serve as an upstream target in the mitophagy pathway regulated by PINK1/Parkin. Metformin can activate the PINK1/Parkinson's disease-dependent mitophagy pathway through SIRT3, counteracting the metabolic imbalance of synthesis and degradation in osteoarthritic chondrocytes induced by IL-1 $\beta$ .<sup>328</sup> Therefore, the mitophagy pathway that Parkinson's relies on plays a crucial part in hindering the advancement of OA (Table 3). Tang et al caused cell damage in primary rat articular chondrocytes by exposing them to advanced glycation end products (AGEs). They discovered that exosomes from bone marrow stem cells (BMSCs) increased the levels of proteins related to autophagy LC3-II/LC3-I and Beclin-1, and enhanced autophagy in chondrocytes that had been treated with AGEs. The increased levels of Drp1 enhance cell apoptosis by inhibiting the expression of proteins related to autophagy, including Beclin-1 and LC3-I/LC3-II. Exosomes obtained from bone marrow mesenchymal stem cells (BMSCs) can counteract the suppression of autophagy caused by the increased levels of Drp1. As a result, BMSC exosomes influence mitochondrial autophagy through DRP1 to inhibit chondrocyte apoptosis, making it a potential target for OA treatment.<sup>329</sup> These outcomes suggest that improving mitophagy in osteoarthritis chondrocytes could be a novel approach for preventing and treating OA, and may also provide a valuable target for future drug screening aimed at OA prevention and treatment (Table 3).

## Clinical Application of Cuproptosis Targeted Therapy Strategy in Osteoarthritis

We have gained insights into the alterations in copper balance associated with osteoarthritic conditions, and when combined with the results from CRGs, this offers a potential therapeutic target for addressing these diseases. The balance of copper in the body is typically reestablished in two ways: by using copper chelators to decrease the availability of copper and by using copper carriers to transport copper into cells, thereby raising the intracellular copper levels.<sup>219</sup> Common agents that chelate copper consist of penicillamine, zinc sulfate (ZS), trientine, and ammonium tetrathiomolybdate (ATTM)<sup>330</sup> (Table 4). Copper chelators have the ability to restrain cell growth by attaching to the surplus copper present within the cells. They can also generate a group of ROS through redox reactions to initiate cell apoptosis, specifically target and remove aged chondrocytes, and successfully enhance cartilage formation to address OA.<sup>219</sup> This unique benefit differentiates them from other medications and offers significant opportunities for future advancement. There is still significant potential for the creation of new copper chelating agents, and continued research in this area is highly anticipated. Research indicates that copper ion carriers like Escitalopram,<sup>331</sup> Disulfiram (DSF),<sup>332</sup> and analogs of

**Table 4** The Clinical Application of Cu-Modulating Agents

| Compound Name               | Mechanism   | Effect  |
|-----------------------------|---|---|
| Penicillamine               | Bind to excess copper in cells to inhibit cell proliferation. A cluster of ROS is produced to induce apoptosis  | Selectively targeted removal of senescent chondrocytes and effectively promote chondrogenesis in the treatment of OA        |
| Zinc sulfate                | Bind to excess copper in cells to inhibit cell proliferation. A cluster of ROS is produced to induce apoptosis  | Selectively targeted removal of senescent chondrocytes and effectively promote chondrogenesis in the treatment of OA        |
| Ammonium Tetrathiomolybdate | Bind to excess copper in cells to inhibit cell proliferation. A cluster of ROS is produced to induce apoptosis  | Selectively targeted removal of senescent chondrocytes and effectively promote badate chondrogenesis in the treatment of OA |
| Trientine                   | Bind to excess copper in cells to inhibit cell proliferation. A cluster of ROS is produced to induce apoptosis  | Selectively targeted removal of senescent chondrocytes and effectively promote chondrogenesis in the treatment of OA        |
| Elesclomol                  | Increase cellular copper levels   | Maintain the stability of mitochondria in articular cells   |
| Disulfiram                  | Increase cellular copper levels   | Maintain the stability of mitochondria in articular cells   |
| Cull(atSm)                  | Increase cellular copper levels   | Maintain the stability of mitochondria in articular cells   |
| Cull(gtSm)                  | Increase cellular copper levels   | Maintain the stability of mitochondria in articular cells   |
| HPP@Cu                      | Clear out reactive oxygen species and active nitrogen regulates the polarization of macrophage phenotypes from M1 production of inflammatory factors to M2, and inhibits the production of inflammatory mediators | Reduce cartilage degradation and reduce the production of inflammatory factors  |

Copper(II) (atSm<sup>333</sup> and gtSm<sup>334</sup>) can elevate copper concentrations within cells, especially in the mitochondria (Table 4). At present, copper ion carriers are not commonly used in the treatment of bone and joint conditions. However, it has been noted that copper levels have increased in most cases of arthritis. Carriers of copper ions can enhance the copper levels inside cells and provide unique benefits in comparison to other medications. The advancement and study of “New carriers of copper ions” hold significant promise, and there is a strong anticipation for more research in this area. Moreover, stress in the endoplasmic reticulum can disturb the balance of non-erythrocyte cells and activate the unfolded protein response (UPR). Prolonged or permanent endoplasmic reticulum stress may lead to cell death that is initiated by the UPR. As a result, focusing on endoplasmic reticulum stress alone or disrupting the UPR signal to decrease cartilage cell death could be a promising approach for treating OA in the future.<sup>335</sup> Nanoparticles are often integrated into the contemporary approaches of cartilage tissue engineering to improve chondrocyte function and hold considerable potential for treating degenerative joint diseases.<sup>336</sup> The thermosensitive hydrogel with multi-functional composite properties (HPP@Cu gel) successfully reduces inflammation by neutralizing reactive oxygen and nitrogen species, shifting macrophage polarization from the M1 to M2 phenotype, and suppressing the release of inflammatory mediators. Research using a rat model of OA has demonstrated that these activities can help alleviate cartilage deterioration and decrease the generation of inflammatory substances.<sup>337</sup>

## Strategies for Inhibiting Chondrocyte Apoptosis

Currently, numerous strategies aim to delay or reverse OA, with inhibiting chondrocyte apoptosis being a central research focus. These strategies mainly fall into the following five categories. In the realm of non-coding RNAs, microRNAs (miRNAs), a class of 20–24 nucleotide-long molecules encoded by endogenous genes, regulate downstream pathways by modulating mRNA stability and translation and inhibiting target gene expression.<sup>338</sup> For example, miR-93 suppresses chondrocyte apoptosis by downregulating the TLR4/NF- $\kappa$ B signaling pathway; miR-107 inhibits the NF- $\kappa$ B pathway by targeting TRAF3, reducing AKT/mTOR activity, promoting autophagy, and ultimately inhibiting apoptosis.<sup>339</sup> miR-27a, miR-206, and miR-186 regulate the PI3K/AKT/mTOR and PI3K/AKT pathways to activate autophagy and inhibit apoptosis in OA chondrocytes.<sup>340,341</sup> Additionally, miR-199-3p and DNMT3B protect chondrocytes through targeted gene regulation,

while miR - 1236 promotes chondrocyte apoptosis.<sup>342–345</sup> Long non - coding RNAs (lncRNAs), such as MINCR, lncRNA - p21, and HOTAIR, often regulate chondrocyte apoptosis by suppressing miRNA expression.<sup>346–352</sup> Circular RNAs (circRNAs) interact with miRNAs, modulating target gene expression and influencing chondrocyte apoptosis.<sup>353,354</sup> Phytochemicals, including *Morroniside*, *Loganin*, *Angelica polysaccharide*, and *Genistein*, possess antioxidant, anti - inflammatory, autophagy - promoting, and anti - apoptotic properties.<sup>355–358</sup> They inhibit chondrocyte apoptosis, reduce synovial inflammation, and balance extracellular matrix metabolism by regulating signaling pathways like NF -  $\kappa$ B, ERK1/2, and SIRT1/AMPK.<sup>300,359–367</sup> Traditional Chinese medicine formulas, such as *Danzi kang knee granules*, can promote stem cell proliferation and cartilage differentiation in OA rats and decrease chondrocyte apoptosis rates.<sup>368</sup> Exosomes play a significant role in inhibiting chondrocyte apoptosis. Exosomes derived from human bone marrow mesenchymal stem cells (BMSCs - exo) can promote type II collagen synthesis, regulate glutamine metabolism, reduce inflammatory cytokine levels, and decrease chondrocyte apoptosis.<sup>369,370</sup> Exosome gels alleviate IL - 1 $\beta$ /LPS - induced chondrocyte apoptosis and degeneration by inhibiting NF -  $\kappa$ B and STAT signaling.<sup>371</sup> Proteins and cytokines, such as NRG4, LMP - 1, Dlx5, and DRP1, inhibit chondrocyte apoptosis by regulating signaling axes like MAPK/JNK. Combining them with drug delivery systems, such as hydrogels and exosomes, can enhance treatment safety and efficacy.<sup>27,372–379</sup> Melatonin can regulate TGF -  $\beta$ , MAPK, or NF -  $\kappa$ B signaling pathways, promoting cartilage matrix synthesis, inhibiting chondrocyte apoptosis, and reducing inflammation.<sup>380–382</sup> Biomaterials, such as melatonin - loaded hydrogels, show promise in promoting chondrocyte adhesion, proliferation, and matrix synthesis.<sup>383,384</sup> Although these strategies for inhibiting chondrocyte apoptosis have made progress, large - scale animal experiments and clinical trials are still needed to further verify their effectiveness and safety.

## Discussion

Cell death is essential for maintaining the proper functioning of a healthy human body. OA, a complicated long-term condition, is significantly affected by the process of programmed cell death in cells. Different types of programmed cell death have specific functions in OA. In this review, we first explore the connection between PCD and OA. For instance, the key features of iron-induced ferroptosis include lipid peroxidation and excess iron, which can result in abnormal chondrocyte death by elevating levels of ROS and MMP13, while reducing levels of type II collagen. The activation of the NLRP3 inflammasome can trigger pyroptosis in chondrocytes. Autophagy can slow down the development of OA by influencing the function of chondrocytes. A novel type of cell death caused by copper ions, referred to as cuproptosis, may also contribute to OA. An overload of copper can disrupt the TCA cycle, causing protein toxicity and resulting in the death of chondrocytes. It's important to highlight that nearly all forms of PCD can generate various inflammatory factors, potentially leading to intense inflammatory reactions and worsening OA. Furthermore, we identified several molecules, including NF- $\kappa$ B, Jak, mTOR, and IER1, that are crucial in the regulation of PCD during the progression of OA. NF- $\kappa$ B has a dual function in the apoptosis of cartilage cells, acting to both promote and inhibit the process. mTOR plays a vital role in autophagy, particularly via the PI3K/AKT/mTOR signaling pathway. mTORC1 can suppress autophagy, which helps reduce the symptoms of OA. Inflammatory agents can activate the JAK/STAT signaling pathway, resulting in the death of chondrocytes.

## Conclusions

In conclusion, we think it is important to investigate various forms of PCD and the signaling pathways associated with the progression of OA for the purpose of developing targeted therapies for the condition. Substances that block PCD may be used as treatment options for OA. We strongly believe that, despite numerous challenges, thorough experiments and clinical studies can position PCD as a significant target for creating therapeutic drugs aimed at the causes of OA, while also reducing the likelihood of side effects and other complications as much as possible.

## Data Sharing Statement

Data are contained within the article.

## 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.

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