Regulatory signaling pathways of osteoblast autophagy in periprosthetic osteolysis

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Periprosthetic osteolysis is a difficult-to-treat complication of arthroplasty. The pathological mechanisms of periprosthetic osteolysis are mainly weakened function of osteoblasts and excessive activation of osteoclasts. Many studies have demonstrated that the imbalance between the formation of bone by osteoblasts and the absorption of bone by osteoclasts is the direct cause of osteolytic diseases. Autophagy, as an important self-protective cellular mechanism, has significant effects on the regulation of osteoblast function, such as osteoblast differentiation, proliferation, and apoptosis. Osteoblasts, which play an important role in maintaining bone homeostasis, have attracted increasing attention in recent years. Up till now, Several signaling pathways have been proved to regulate autophagy of osteoblasts, including the AMPK, NF-κB, FoxO3 and other signaling pathways. This article reviews the recent progress in understanding osteoblast autophagy and mitophagy in the context of periprosthetic osteolysis and the signaling pathways which are involved in these processes. By summarizing previous studies describing the mechanism underlying osteoblast autophagy, we wish to contribute new therapeutic ideas and potential therapeutic targets for periprosthetic osteolysis.

KEYWORDS

wear particles, periprosthetic osteolysis, osteoblasts, autophagy, signaling pathways

1 Introduction

As the world ages, the number of patients receiving total joint replacement (TJR) is increasing. As the preferred treatment for end-stage bone and joint diseases, TJR effectively relieves pain and re-establishes joint function, which vastly improving patients' quality of life (Talmo et al., 2010). According to data from 2000 to 2014, the number of total hip arthroplasty (THA) procedures is expected to increase by 71%–635,000 by 2030, and the number of total knee arthroplasty (TKA) procedures is expected to increase by 85%–1,260,000 (Sloan et al., 2018). However, increasing research shows that an increase in the number of TJRs inevitably leads to a series of postoperative complications, such as infection, periprosthetic fracture, instability/dislocation, neurovascular injury, periprosthetic osteolysis (PPOL), and so on (Talmo et al., 2012). Most complications can be prevented through effective preoperative examination and high-quality postoperative care, but PPOL is a difficult problem for doctors and patients (Purdue et al., 2007). Among all reported joint replacement operations, the hazards posed by PPOL are common and serious. According to data from the National Joint Replacement Registration System of the United Kingdom, PPOL most often leads to failed hip and knee revision surgeries, with

approximately 48.1% of hips needing hip revision and 39.6% of knees needing revision (Blom et al., 2021). Although PPOL has been studied for more than 50 years, its pathogenesis has not been fully clarified. Although some therapeutic methods have led to positive results in experimental animal models, few cases have been successfully treated in clinical practice. Therefore, more research and investment are still needed for PPOL prevention and treatment. Over time, wear debris generated on the surface of a prosthetic joint is the main factor limiting the survival of joint implants. Considerable research-based evidence has shown the promoting effect of chronic low-grade inflammation on PPOL. Many experiments have repeatedly shown that after artificial prosthesis wear particles contact immune cells, the latter express proinflammatory factors, chemokines and other substances. This cellular response results in chronic aseptic inflammation of surrounding tissue (Ollivere et al., 2012). In addition, many osteoclasts are generated due to inflammation, and the osteogenic capacity of osteoblasts is reduced due to inflammation. Multiple factors promote each other to form the pathophysiological basis of PPOL; however, the main cause is local bone metabolism imbalance caused by the functional disorder of osteoblasts and osteoclasts (Noordin and Masri, 2012).

Osteoblasts adhere to the bone surface after differentiation from mesenchymal stem cells (MSCs). Their functions include mainly generating bone matrix and regulating osteoclast functions. They promote mineralization of bone matrix by synthesizing type I colloidin (Kim et al., 2020). In an osteolytic microenvironment influenced by wear particles, the osteoblast phenotype is changed, with the expression of corresponding genes changing, diminishing the osteogenic capacity of osteoblasts (Vermes et al., 2001). The literature suggests that the damage to osteoblast osteogenic ability induced by wear particles and the reduction in collagen synthesis are direct causes of PPOL occurrence and development. Besides, the inflammation and oxidative stress induced by wear particles can directly injure osteoblasts and thus lead to a reduction in their number (O'Neill et al., 2013).

Autophagy can help cells survive nutritional stress at critical times of development. When cells are in a state of stress or their internal nutrient and energy needs are increased or reduced by other autophagy-inducing factors, the autophagy in cells is activated and contributes to cell adaptation to the new environment (Dikic and Elazar, 2018). In addition, autophagy plays a "housekeeping" role by eliminating misfolded proteins, damaged organelles and intracellular pathogens (Kim and Lee, 2014). Autophagy can be further activated under stress conditions such as nutrient or energy deficiency to degrade cytoplasmic substances into metabolites that can be used for biosynthesis or energy production to promote cell survival. Therefore, Moderate autophagy has a protective effect on cells, while excessive autophagy may be harmful and cause irreversible cell damage (Maiuri et al., 2007). Autophagy is regulated by a group of genes which called autophagy-related genes (ATGs). Autophagy is mediated after the formation of an "autophagosome", which is an isolated double membrane transiently formed organelle (also known as a phagocyte). Excess or potentially damaging cytosol is isolated in an autophagosome and delivered to a lysosome, where it is degraded (Mizushima et al., 2008). Autophagy is an effective means by which osteoblasts protect themselves in harmful environments and plays a key role in

maintaining the balance of bone metabolism, osteoblast proliferation and differentiation and bone formation (di Giacomo et al., 2020). However, excessive autophagic flux leads to the death of osteoblasts, and inhibiting excessive autophagic flux is thus a potential strategy to treat osteolysis (Ma et al., 2021). Proanthocyanidins can be used to treat PPOL in mouse skulls caused by tricalcium phosphate (TCP) wear particles by inhibiting autophagy and oxidative stress (Lin et al., 2019). Zhiwei Zhang et al. used nano alumina (Al) particles and bortezomib (BTZ) to reduce the rate of osteoblast autophagy induced by titanium (Ti) particles and thereby attenuate cell apoptosis, inflammation and osteolysis (Zhang Z. et al., 2020). Liu N, Jia QiYan and others also proved that inhibition of autophagy can be an efficient treatment for peri-implant osteolysis induced by stress granules (Liu N. et al., 2016; Yan et al., 2018).

Classical autophagy is a process involving the sequential and selective activation of multiple ATGs proteins. Initiation of the autophagy cascade is physiologically inhibited by MTORC1, which can mediate the inactivation phosphorylation of ATG13 and ULK1. The complex containing ULK1 and ATG13 is central to initiating autophagy (Klionsky et al., 2021). AMPK can counteract the inhibition of MTORC1 by phosphorylating ULK1 and Beclin1. ULK1 triggers an autophagy cascade by promoting Beclin1 to form complexes with different proteins (Galluzzi et al., 2017). In addition, multiple signaling pathways and transcription factors have been shown to regulate ATGs expression. These different signaling pathways together constitute a complex autophagy network.

Therefore, an in-depth understanding of the mechanisms of autophagy is needed for developing treatments for PPOL. This article mainly reviews the effects of osteoblast autophagy in PPOL and related signal transduction pathways with the aim of suggesting new ideas and potential therapeutic targets for PPOL. Figure 1 shows the major effects of autophagy on osteoblasts in PPOL. Table 1 lists important studies from the past decade on autophagy in osteoblasts during PPOL. Table 2 summarizes some key autophagy genes or signaling pathways that play an important role in regulating the function of osteoblasts.

2 Autophagy regulates the function of osteoblasts in the context of PPOL

2.1 Dual regulation of autophagy on osteoblast apoptosis

The apoptosis of osteoblasts induced by wear particles is recognized as a cause for the occurrence and progression of osteolysis near a prosthesis, mainly due to chronic inflammation and oxidative stress induced by the wear particles (Zhang Y. et al., 2022). This process may be reversed by enhancing the antiapoptotic ability of osteoblasts. All cells respond quickly to the external environment or conditions by regulating autophagic flux to survive stress conditions. Survival stress mainly includes a high sugar level, a low oxygen level, inflammation, hunger, hormone activity, and oxidative stress (Lacarrière-Keïta et al., 2022). Jia J et al. demonstrated that autophagy may contribute to osteoblast survival
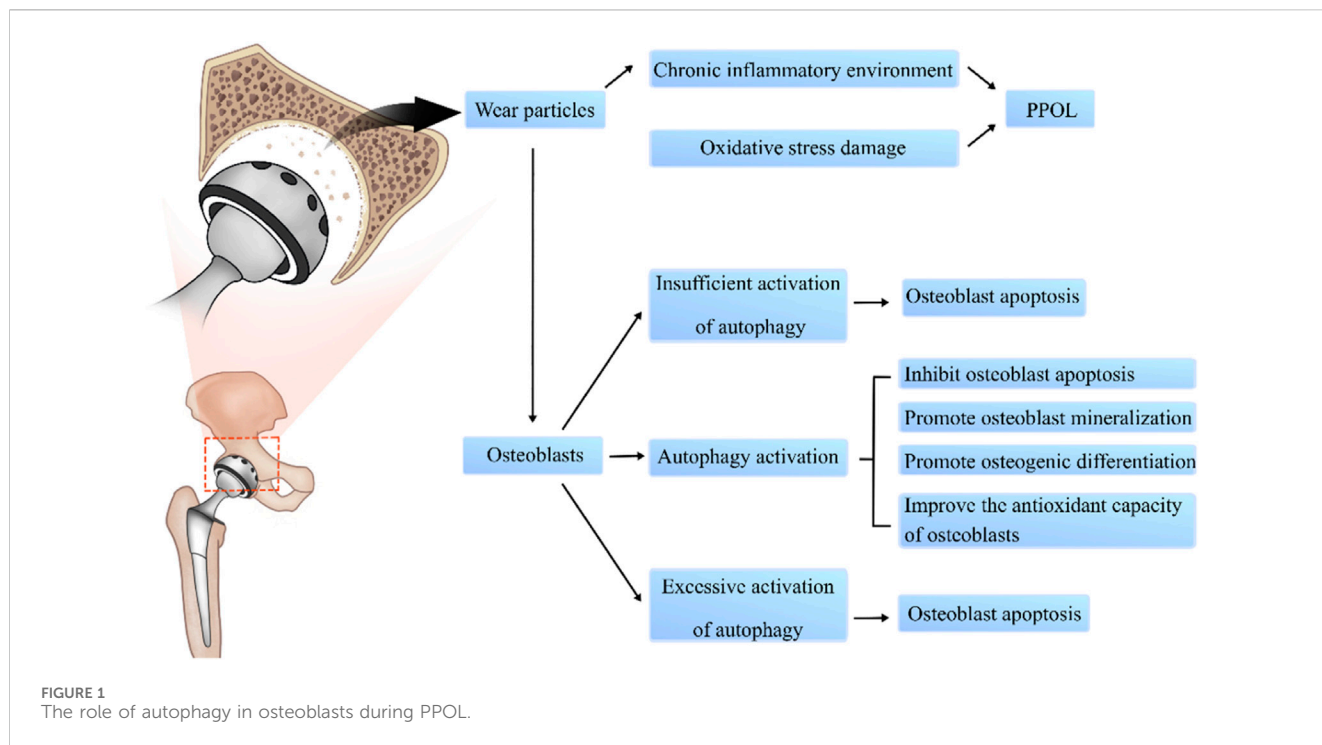
FIGURE 1
The role of autophagy in osteoblasts during PPOL.

TABLE 1 Summary of important studies from the past decade on autophagy in osteoblasts during osteolysis.

Intervention factor	Autophagy state	Downstream signaling	Effect of autophagy on osteoblasts	Refs
Low Doses of Glucocorticoids	Active	Atg7, LC-3α, LC-3β	Apoptosis inhibition	Yao et al. (2013)
CoCrMo Metal Particles	Active	ERN1-MAPK8	Apoptosis activation	Wang et al. (2015)
Porous Tantalum	Active	LC3-II, p62	Proliferation activation	Kang et al. (2017)
Simvastatin	Active	LC3-II, p62, Beclin1	Osteogenic differentiation activation	Xu R. et al. (2018)
Galangin	Active	p62, Beclin1, Atg5, LC3A	Osteogenic differentiation activation	Kim et al. (2018)
Oncostatin M Receptor Overexpression	Inhibit	SHC1, ERK1/2	Osteogenic differentiation inhibition	Zhou et al. (2022)
Atg7 Knock Down	Inhibit	LC3-II, p62	Mineralization inhibition and Apoptosis activation	Li H. et al. (2018)
Atg5 Knock Down	Inhibit	LC3-II, BECN1, Atg7, Atg5	Mineralization inhibition	Nollet et al. (2014)

under stress conditions after glucocorticoid exposure. Lower concentrations of glucocorticoids can activate autophagy in osteoblasts, while higher concentrations of glucocorticoids induce apoptosis (Jia et al., 2011). Xia et al. showed that certain metabolic stressors can activate autophagy in osteoblasts. Autophagy may be a mechanism by which bone cells repair damaged organelles or cell membranes. However, sustained high levels of stress can lead to the accumulation of autophagosomes and cell death (Xia et al., 2010). Therefore, the activation of autophagy induced by stress factors alone is attenuated but not enough to overcome sustained high stress levels. Can we enhance the antiapoptotic capacity in osteoblasts under survival stress through artificial autophagy regulation? Recent reports suggest that β-ecdysterone regulates autophagy in

osteoblasts. Specifically, β-ecdysterone reverses dexamethasone-induced osteoblast apoptosis by promoting autophagy in osteoblasts through the AMPK/mTOR pathway. Moreover, β-ecdysone-induced autophagy activation has been associated with the promotion of osteoblast differentiation and fracture healing (Tang et al., 2021). Similarly, studies have shown that paeoniflorin inhibits dexamethasone-induced osteoblast apoptosis by enhancing autophagic flux in osteoblasts by regulating the AKT/mTOR signaling pathway (Yang et al., 2021).

However, other studies have shown that the application of autophagy inhibitors attenuated CoCrMo metal particle-induced osteolysis near prostheses. CoCrMo metal granule-induced autophagy in osteoblasts may increase the expression of the

TABLE 2 Summary of important studies in the past decade on osteoblast autophagy in the context of osteolysis.

Signaling Pathways/Genes	Autophagy state	Effect on osteoblasts	Refs
AMPK/mTOR/ULK1	Active or Inhibit	Maintain cell stability	Ran et al. (2020)
NF-κB	Active or Inhibit	Regulate key ATGs	Zheng et al. (2020)
FOXO3	Active	Activate autophagy or apoptosis	Gómez-Puerto et al. (2016)
Beclin1/Bcl-2	Active	Mature the autophagosome	Wu et al. (2021)
p62	Active	Transport proteins to lysosomes	Puissant et al. (2012)
Sirt1	Active	Regulate key ATGs	Jang et al. (2012)
ERK1/2	Active	Activate autophagy or apoptosis	Dagda et al. (2008)
Pink1/Parkin	Active	Regulate mitochondrial autophagy	Zhang W. et al. (2020)

apoptotic proteins BAX and CASP3, thereby exacerbating osteolysis (Wang et al., 2015). In some cases where autophagy upregulation is uncontrolled, overactivation of autophagy can be lethal to cells. This may be due to stress exceeding the extent to which autophagy can compensate, or to the inability of cells to survive non-specific degradation of large amounts of cytoplasmic contents. Overexpression of Atg1 in *Drosophila* is sufficient to induce high levels of autophagy, which has the ability to inhibit cell growth and induce apoptosis (Scott et al., 2007). Bcl-2, as an anti-apoptotic protein, can interact with the evolutionarily conserved autophagy protein Beclin1. Blocking the binding of Bcl-2 to Beclin1 induces excessive autophagy and promotes apoptosis. Bcl-2 acts not only as an anti-apoptotic protein, but also as an anti-autophagy protein through its inhibitory interaction with Beclin1 (Pattingre et al., 2005). Therefore, the regulation of autophagy can increase antiapoptotic activity in osteoblasts, showing certain potential research value for the treatment of PPOL. Autophagy has two sides: it increases the antiapoptotic capacity of normal cells by degrading damaged organelles and recycling energy-related molecules, but excessive self-phagocytosis induces osteoblast apoptosis.

2.2 The dual role of autophagy in osteogenic differentiation

BMSCs are the main sources of osteoblast differentiation. They can differentiate into bone, cartilage, fat, nerve and muscle cells (Ning et al., 2022). MC3T3-E1 precursor osteoblasts are the main cells used in osteogenic differentiation studies performed *in vitro* (Ren et al., 2023). The development of the chronic inflammatory environment induced by wear particles can inhibit the differentiation of BMSCs into osteoblasts and promote the differentiation and proliferation of osteoclasts. This effect shifts the bone balance toward bone resorption and ultimately leads to increased osteolysis (Deng et al., 2021).

Activation of autophagy may reverse the inhibitory effect of abrasive particles on osteogenic differentiation. Resveratrol induces osteogenic differentiation of MSCs by promoting autophagy and synergizing with osteogenesis-inducible factors (Vidoni et al., 2019). Strontium (Sr) at a concentration of 3 mM activated the AMPK/mTOR signaling pathway to promote the differentiation of MC3T3-

E1 cells into osteoblasts, and inhibition of autophagy counteracted this effect (Cheng et al., 2019). During the differentiation of BMSCs into osteoblasts, inhibition of OSMR expression activates the ERK1/2 signaling pathway and induces autophagy, thus facilitating this process (Zhou et al., 2022).

Many scholars support that autophagy activation promotes the differentiation of osteoblasts, which contributes to alleviating osteolysis near prostheses. However, it has also been reported that the effect of autophagy on osteoblast differentiation is bidirectional. As a traditional Chinese medicine formulation, QiangGuYin activates the AKT/mTOR pathway and inhibits autophagy in osteoblasts by inhibiting the expression of CIP-1, thereby attenuating osteoporosis (Yuan et al., 2022). AMPK is a major pathway leading to autophagy activation. Notably, the preliminary activation of AMPK is necessary for osteoblast differentiation. The continuous activation of the AMPK or autophagy pathway in the late stage of osteoblast differentiation has inhibitory effects (Xi et al., 2016). Pantovic A et al. demonstrated that osteogenic differentiation of BMSCs requires the initial activation of the AMPK/mTOR autophagy pathway and later activation of the Akt/mTOR autophagy pathway (Pantovic et al., 2013). Therefore, the regulation of autophagy plays a very important role in the differentiation of osteoblasts, and further study of its regulatory mechanism may lead to a new treatment for osteolysis.

2.3 Autophagy enhances the antioxidative stress response in osteoblasts

Ti granule-induced oxidative stress injury in osteoblasts has been demonstrated (Wei et al., 2009). Ti particles mediate mitochondrial damage in osteoblasts. When mitochondria are damaged, they produce too much excessive reactive oxygen species (ROS). Among the ROS targets are mitochondria themselves, and a vicious cycle develops between ROS and mitochondria. The main effect of oxidative stress after bone injury is inhibited function and activity of osteoblasts and bone cells and inhibited bone formation. Moreover, oxidative stress promotes osteoclast proliferation and enhances bone resorption (Hameister et al., 2020). These discoveries indicated that ROS overexpression exacerbates wear granule-induced osteolysis, and maintaining nromal mitochondrial function is necessary for the

function and activity of osteoblasts. As an important mechanism contributing to mitochondrial quality maintenance, mitochondrial autophagy can mediate the degradation of damaged mitochondria and reduce the production of ROS, thereby protecting other normal cells and maintaining mitochondrial homeostasis (Shi et al., 2023).

Although not effective in all cells, resveratrol has been shown to function as an autophagy activator in osteoblasts. Xuhao Yang et al. found that the application of resveratrol to osteoporotic rats enhanced mitophagy in osteoblasts by increasing the expression of Sirt1, thereby attenuating the oxidative stress-induced damage to osteoblasts induced by glucocorticoids. (Yang et al., 2019). Bartolome A et al. demonstrated that autophagy inhibition in osteoblasts exacerbated oxidative stress damage in a high-glucose environment (Bartolomé et al., 2013). Low doses of glucocorticoids promoted autophagy in osteoblasts in response to hormone-induced oxidative stress damage. When the hormone dose was increased, autophagy attenuated the increase in ROS levels, and osteoblasts underwent apoptosis (Zhang et al., 2018).

In addition to the activation of mitochondrial autophagy, autophagy can also directly regulate cellular antioxidant defense mechanisms. The most relevant interaction between autophagy and oxidative stress response mechanisms is achieved through Nrf2 signaling. When autophagy is activated, the accumulated p62 can compete with Keap1 to activate Nrf2. This results in a reversal of Keap1 binding to Nrf2. The continuous accumulation of non-ubiquitinated Nrf2 can mediate the activation of antioxidant gene transcription (Lee et al., 2020). Besides, the activation of Nrf2 can also have a positive effect on the autophagy process. NDP52 is a cargo recognition protein regulated by NRF2. NDP52 acts in a similar way to p62, mainly mediating the degradation of goods in lysosomes (Jo et al., 2014). Moreover, NRF2 can regulate other autophagy related genes, such as ULK1, p62, ATG7, ATG4 and so on (Pajares et al., 2016). SIRT1 has also been proved to activate antioxidant defense mechanisms by mediating the deacetylation of Nrf2 in vitro (Ding et al., 2016). Foxo3 also shows a similar role as a transcription factor, which can activate antioxidant defense mechanisms by regulating the level of autophagy in cells. Therefore, there is a close relationship between the signaling pathway of autophagy and Reduction-Oxidation.

In conclusion, autophagy activation may confer self-protection to osteoblasts in response to oxidative stress injury. However, the level of autophagy induced by survival stress is limited, and cells struggle to mitigate progressively enhanced oxidative stress damage. Therefore, targeted regulation of autophagy to increase the antioxidative capacity of osteoblasts is important for improving the prognosis of osteolysis.

2.4 The dual role of autophagy in osteoblast mineralization

The mineralization of osteoblasts has great effects in the prognosis of osteolysis near a prosthesis. Osteoblast mineralization is a complex process involving many signaling pathways and cellular processes (Hasegawa et al., 2022). Autophagy has been recently identified as a necessary condition for the mineralization of osteoblast. Osteoblast mineralization itself can induce autophagy. Autophagosomes can carry secreted apatite
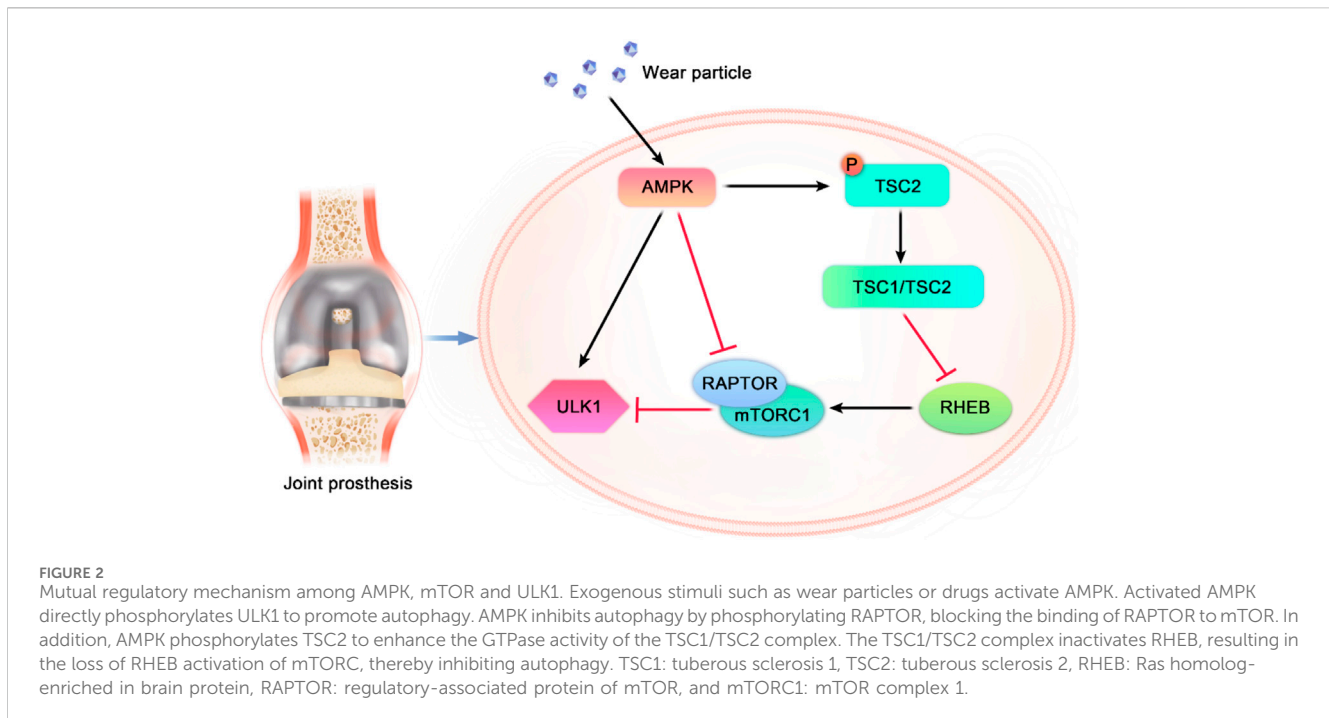
crystals to the extracellular space. Atg5-deficient transgenic mice showed a significant decrease in bone mass that was mainly attributed to a decreased number of osteoblasts and thus decreased mineralization (Nollet et al., 2014). As an autophagy-activating pathway, the AMPK pathway promotes the differentiation and mineralization of osteoblasts by inducing autophagy. The application of AMPK pathway inhibitors reverses this effect (Li Y. et al., 2018). Vitro studies prove that kaempferol enhances the mineralization ability of MC3T3-E1 cells by promoting autophagy (Kim et al., 2016). Vivo studies suggest that glucosamine induces osteoblast differentiation and mineralization by promoting autophagy, thereby improving the prognosis of osteoporotic mice (Su et al., 2022). However, other studies show that rapamycin-induced autophagy significantly reduces the activity and mineralization of osteoblasts. While overexpression of RUNX2 reverses this result by inhibiting ATGs expression (Ren et al., 2022). Therefore, autophagy exerts different effects on osteoblast mineralization according to the different pathways it activates and the degree to which they are activated. Autogenerated autophagy in cells challenged with environmental stress is generally considered beneficial, but excessive autophagic flux induced by certain pathological factors, including external stimuli, may impair cell activity and function.

In conclusion, autophagosomes are carriers of osteoblast mineralized crystals and are necessary conditions for osteoblast mineralization. Unfortunately, the mechanism by which autophagy regulates osteoblast mineralization remains unclear, mainly due to the complexity of the mineralization process and poorly characterized autophagy signaling pathways.

3 The autophagy signaling pathway in osteoblasts

3.1 The AMPK/ULK1/mTOR pathway

AMPK is an AMP-dependent protein kinase. It is a heterotrimeric complex composed by α, β and γ subunits. AMPK activation increases the catabolism (ATP production) pathway activation rate and reduces the anabolic (ATP utilization) pathway activation rate. Thus, AMPK has great effects in whole-body energy metabolism (Carling, 2017). In decades, AMPK has been identified as the guardian of mitochondrial homeostasis (Friis et al., 2014). AMPK induces autophagy through two different mechanisms: inhibiting the activation of mammalian rapamycin target protein (mTOR) and inducing the direct phosphorylation of mammalian autophagy initiation kinase (ULK1). In addition, mTOR and ULK1 interact. Therefore, AMPK, mTOR and ULK1 activate or inhibit each other to form a triangular network (Alers et al., 2012). AMPK is phosphorylated when an organism has a low energy level or is hungry. Activation of AMPK can induce autophagy by inhibiting mTOR activity in two ways: (1) AMPK enhances the activity of TSC1/TSC2 complex by mediating the phosphorylation of TSC2. The TSC1/TSC2 complex inactivates RHEB by hydrolyzing GTP. Inactivated RHEB can not induce the activation of mTORC1. (2) AMPK can block RAPTOR binding to mTOR or mTOR substrates and inhibiting the activation of mTOR signaling pathways by directly

FIGURE 2
Mutual regulatory mechanism among AMPK, mTOR and ULK1. Exogenous stimuli such as wear particles or drugs activate AMPK. Activated AMPK directly phosphorylates ULK1 to promote autophagy. AMPK inhibits autophagy by phosphorylating RAPTOR, blocking the binding of RAPTOR to mTOR. In addition, AMPK phosphorylates TSC2 to enhance the GTPase activity of the TSC1/TSC2 complex. The TSC1/TSC2 complex inactivates RHEB, resulting in the loss of RHEB activation of mTORC, thereby inhibiting autophagy. TSC1: tuberous sclerosis 1, TSC2: tuberous sclerosis 2, RHEB: Ras homolog-enriched in brain protein, RAPTOR: regulatory-associated protein of mTOR, and mTORC1: mTOR complex 1.

phosphorylating RAPTOR. The inactivation of mTOR reduces the inhibitory effect of mTOR on ULK1. Then AMPK binds to ULK1 and induces autophagy (Li and Chen, 2019). In addition, AMPK directly phosphorylates multiple sites in ULK1 to increase autophagic flux (Kim et al., 2011). In conclusion, both AMPK and mTOR regulate ULK1 function. An increasing number of reports have shown two-way regulation among AMPK, mTOR and ULK1, which means that AMPK, ULK1 and mTOR form a signal triplet. They regulate each other to maintain dynamic autophagy in balance (Wong et al., 2013). Figure 2 shows a schematic diagram of the AMPK/mTOR/ULK1 signaling pathway.

The ability of AMPK to regulate autophagy can affect the function and activity of osteoblasts in osteolytic diseases. Negative pressure drainage at a wound site enhances autophagy in osteoblasts by activating AMPK and regulating ULK1 function, thus promoting bone regeneration (Zhang S. et al., 2022). Through the osteogenic differentiation of human dental pulp MSCs, knocking down AMPK prevented early mTOR inhibition and weakened autophagic flux, which hindered osteoblast differentiation. The inhibition of Akt reduces the mTOR activation rate, enhances autophagic flux and promotes osteoblast differentiation (Pantovic et al., 2013). Inhibition of Akt reduces mTOR activation and enhances autophagy, which can promote osteoblast differentiation (Lu et al., 2021).

Under physiological conditions, autophagic flux in osteoblasts remains at the basal level. When cells encounter excessive harmful stimuli such as wear particles or oxidative stress and inflammation-inducing stimuli, autophagic flux is increased to enhance self-protection. However, typically, the autophagic flux in osteoblasts is insufficient to attenuate the response to foreign stimuli, resulting in osteolysis. The AMPK signaling pathway is a main pathway that enhances autophagic flux in osteoblasts challenged with wear particles. The upregulation of AMPK leads to inhibited mTOR activation and promoted ULK1 activation, both of which increase autophagic flux in osteoblasts and thus confer self-protection to osteoblasts challenged with harmful stimuli. Therefore, targeted activation of AMPK to promote autophagy has become a potential treatment for osteolysis near prostheses.

3.2 NF-κB

Nuclear factor κB (NF-κB) family members and the retroviral oncoprotein v-Rel constitute the NF-κB/Rel protein family, which has an important position in inflammation and the immune response (Sun, 2017). NF-κB is formed by the dimerization of five subunits, namely, NF-κB1 (p50), NF-κB2 (p52), RELA (also known as p65), RELB and c-REL (Hayden and Ghosh, 2008). NF-κB activation is mediated through two main signaling pathways: the classical and nonclassical NF-κB signaling pathways. The classical signaling pathway mediates the activation of NF-κB1 (p50), RELA and c-Rel, while the nonclassical signaling pathway mediates the activation of NF-κB2 (p52) and RELB (Sun, 2011). As the NF-κB pathway is activated by interleukin-1 (IL-1) and tumor necrosis factor-α (TNFα) and the NF-κB pathway exerts effects on the expression of other proinflammatory genes, the NF-κB pathway is considered a typical proinflammatory signaling pathway (Lawrence, 2009). Moreover, the antiapoptotic effect of NF-κB shows that it plays a crucial role in the occurrence and development of tumors (Hoesel and Schmid, 2013). Complex crosstalk between the NF-κB signaling pathway and components of the autophagy pathway has been reported (Verzella et al., 2020). Depending on the different inducers, NF-κB can positively or negatively regulate autophagy activation. Mojgan Djavaheri-Mergny et al. showed that TNFA-induced NFkB activation leads to inhibition of autophagy in cell lines derived from three types of

cancer (Ewing sarcoma, breast cancer and acute promyelocytic leukemia) (Djavaheri-Mergny et al., 2007). Role of hypoxia et al. showed that pharmacologically activated NF-κB under hypoxia conditions can induce autophagy in stromal fibroblasts (Martinez-Outschoorn et al., 2010). Studies by Mathieu Nivon et al. have shown that NF-κB can activate autophagy during the recovery phase of cellular heat shock response and increase cell survival by clearing irreversibly damaged proteins (Nivon et al., 2009). NF-κB can also affect autophagy by inducing or inhibiting the expression of multiple ATGs. NF-κB and its family members p65/RelA recognize and bind the promoter of Beclin1, promoting the expression of the Beclin1 gene and enhancing autophagy. Interestingly, NF-κB exerts the opposite regulatory effect on Beclin1. Activation of NF-κB upregulates the expression of A20, which inhibits Beclin1 ubiquitination and thus inhibits autophagy (Trocoli and Djavaheri-Mergny, 2011). In addition, NF-κB influence autophagy by regulating Bnip3. Under physiological conditions, p65 inhibits Bnip3 transcription and reduces autophagy by preventing E2F1 from binding to the Bnip3 promoter. The activity of NF-κB decreases under hypoxic conditions, which leads to increased Bnip3 expression and autophagy induction (Shaw et al., 2008). It has also been suggested that starvation can induce multiple sites phosphorylation of Bcl-2 by JNK1 signaling pathway and mediate the dissociation of Bcl-2/Beclin1 complex. The NF-κB signaling pathway can inhibit the dissociation of the Bcl-2/Beclin 1 complex by inhibiting the JNK1 signaling pathway, thereby inhibiting autophagy (Wei et al., 2008) The regulatory effect of NF-κB on autophagy is briefly shown in Figure 3.

The NF-κB signaling pathway also promotes autophagy in osteoblasts and has significant effects in regulating the function and activity of osteoblasts (Zhang et al., 2021). Without drug stimulation or other interventions, osteoblasts exhibit a certain level of basic autophagic flux. SN50, a blocking agent of the NF-κB pathway, exerts a significant effect on autophagy in osteoblasts only when administered at a certain concentration (25 mg/mL). At this dose, it significantly inhibited autophagic flux in osteoblasts by blocking the NF-κB pathway (Qin et al., 2016). Tnf-α promoted osteoblast autophagy and reduced osteoblast apoptosis in avascular necrotic tissue in the femoral head by activating the P38 MAPK/NF-κB signaling pathway (Zheng et al., 2020). Low concentrations of lipopolysaccharide (LPS) promoted osteoblast autophagy and osteoblast proliferation and inhibited osteoblast apoptosis by activating the NF-κB signaling pathway, thereby accelerating fracture healing (Xu M. X. et al., 2018). However, other studies have shown that diosgenin enhances the differentiation and proliferation of osteoblasts by inhibiting osteoblast autophagy through the ASPP2/NF-κβ pathway (Zhu et al., 2017).
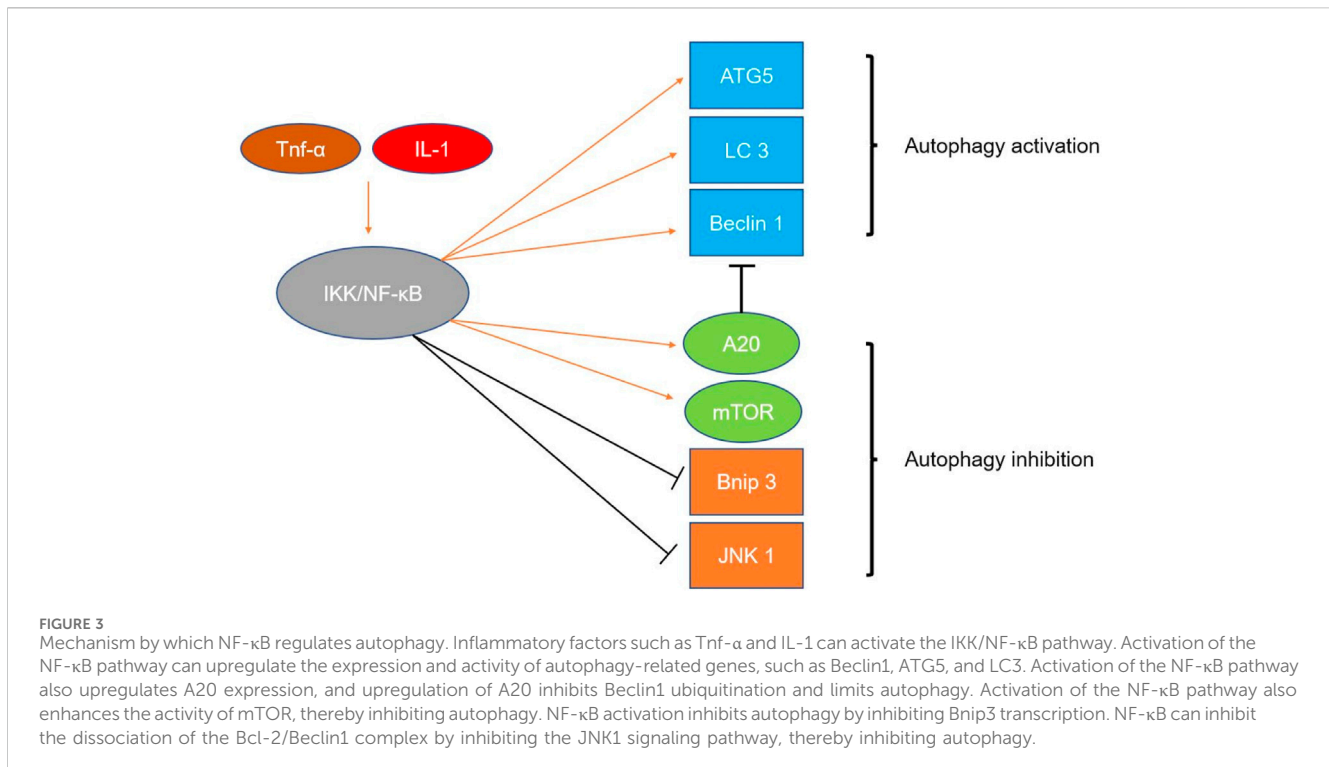
As a classical inflammatory pathway, the NF-κB pathway engages in complicated crosstalk with autophagy and inflammatory pathway components. Interference with NF-κB activity under different conditions may exert different effects on osteoblast activity and function. The regulatory effect on autophagy mediated by NF-κB suggests a mechanism that links inflammation and immune signaling to cell death and self-phagocytosis, but the exact mechanism is unclear. The upregulation of IL-1 and TNF-α induced by wear particles exerts a direct activating effect on the NF-κB pathway. Therefore, the regulation of autophagy by NF-κB plays

an important role in the development and outcome of PPOL. Although NF-κB knockout inhibits apoptosis by reducing inflammation, it also abrogates the protective effect of NF-κB-mediated autophagy. Therefore, the regulation of the NF-κB pathway is important to the prognosis of osteolysis near a prosthesis.

3.3 FOXO3

In mammals, four members of the FoxO family are expressed: Foxo1, FoxO3, Foxo4 and Foxo6. FoxOs were initially identified as downstream regulators of the insulin pathway. FoxO can control several processes critical to cell homeostasis by regulating multiple targeted genes, including cellular energy production, resistance to oxidative stress, and cell viability and proliferation (Link, 2019). The activity of the FoxO transcription factor can be inhibited by insulin and growth factor signals. Acute loss of IR/IGF1R in the muscles of diabetic rats left FoxOs uninhibited, and the mitochondrial respiration mediated by complex I was thus strongly inhibited (Bhardwaj et al., 2021). Although the FoxO family shows the ability to induce autophagy, the role played by FoxO3 is the most important to autophagy. FoxO3 has long been considered to play a key role in the molecular basis of longevity (Morris et al., 2015). In response to cell survival stress, FoxO3 is transferred to the nucleus and induces the expression of multiple ATGs, including LC3b, Gabarapl1, PI3KII, Ulk1, Atg12, Beclin1, Atg4b, and Bnip3, to promote autophagy. TLR4 (Toll-like receptor 4) inhibits autophagy in microglia through the PI3K-FoxO3 pathway, thereby reducing the phagocytosis rate (Lee et al., 2019). Recently, FoxO3 was shown to downregulate apoptosis by upregulating autophagy in osteoblasts (Fitzwalter and Thorburn, 2018). In MSCs, FoxO3 needs to be activated to induce autophagy, which attenuates any increase in ROS levels caused by increased mitochondrial respiration during osteoblast differentiation and reduces the osteoblast apoptosis rate. Gomez Bodo et al. found that during the osteogenic differentiation of human BMSCs, mitochondrial metabolism is accelerated to produce enough energy to drive differentiation, which leads to an increase in ROS levels and FoxO3 activation. This process depends mainly on Ser294 phosphorylation in FoxO3 induced by mitogen-activated protein kinase 8 (MAPK8). These findings show that FoxO3 activation is important for reducing ROS levels by activating autophagy (Gómez-Puerto et al., 2016).

What's more, it is reported that FoxO3a is part of the autophagy homeostasis feedback monitoring loop. In cases when normal autophagy of cells is inhibited, FoxO3a induces apoptosis if appropriate levels of autophagy are not re-established within a certain period. The relationship between FoxO3a and autophagy and apoptosis is briefly shown in Figure 4. The transcription factor FoxO3a is both a regulator and a substrate of autophagy. Under physiological conditions, FoxO3a is degraded by basal autophagy. The destruction of autophagy leads to the accumulation of FoxO3a, which leads to the upregulation of BBC3/PUMA, thus inducing apoptosis (Fitzwalter et al., 2018). FoxO3a, as a coinducer of autophagy and apoptosis, is associated with autophagy and apoptosis. FoxO3a induces autophagy by reversely activating autophagy-related genes when autophagy levels are inhibited. However, under the continuous inhibition of autophagy, FoxO3a can induce cell apoptosis as well (Fitzwalter and Thorburn, 2018). In

FIGURE 3
Mechanism by which NF-κB regulates autophagy. Inflammatory factors such as Tnf-α and IL-1 can activate the IKK/NF-κB pathway. Activation of the NF-κB pathway can upregulate the expression and activity of autophagy-related genes, such as Beclin1, ATG5, and LC3. Activation of the NF-κB pathway also upregulates A20 expression, and upregulation of A20 inhibits Beclin1 ubiquitination and limits autophagy. Activation of the NF-κB pathway also enhances the activity of mTOR, thereby inhibiting autophagy. NF-κB activation inhibits autophagy by inhibiting Bnip3 transcription. NF-κB can inhibit the dissociation of the Bcl-2/Beclin1 complex by inhibiting the JNK1 signaling pathway, thereby inhibiting autophagy.

summary, FoxO3a is essential for the regulation of autophagy homeostasis and the regulation of normal physiological functions of cells.
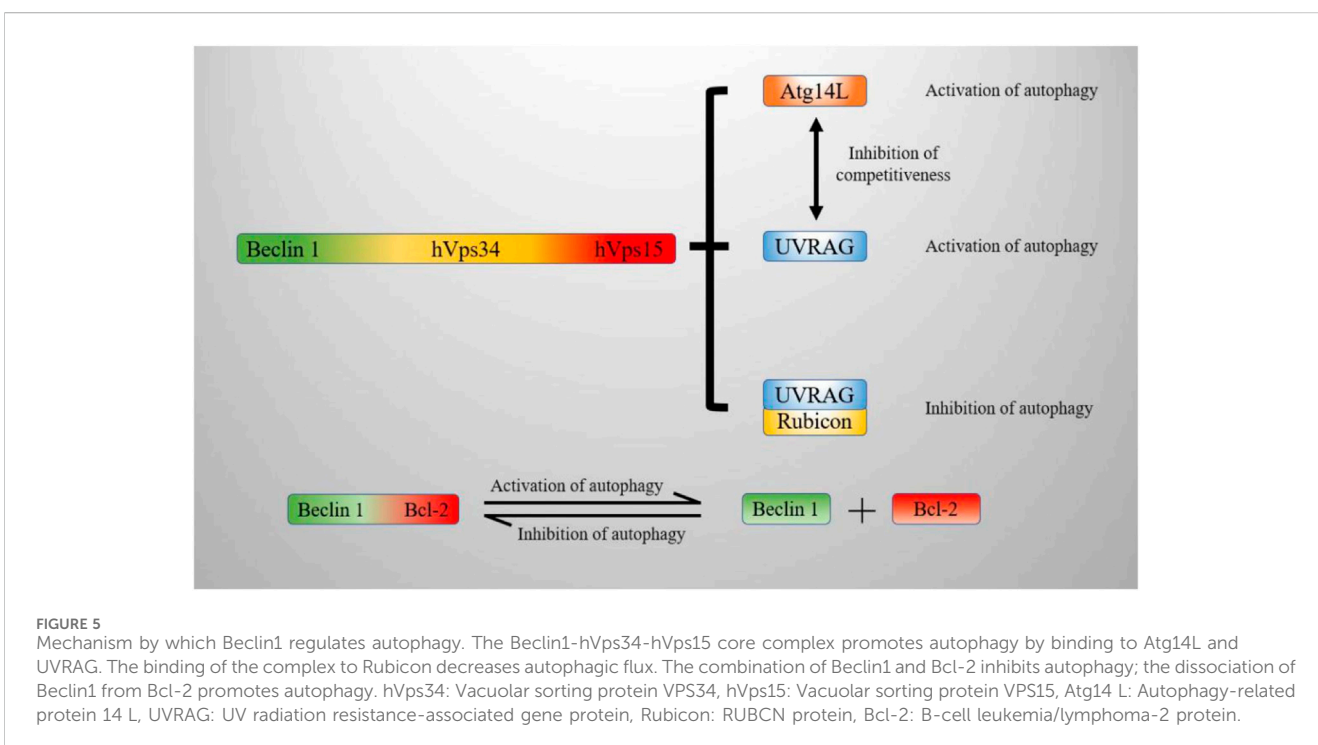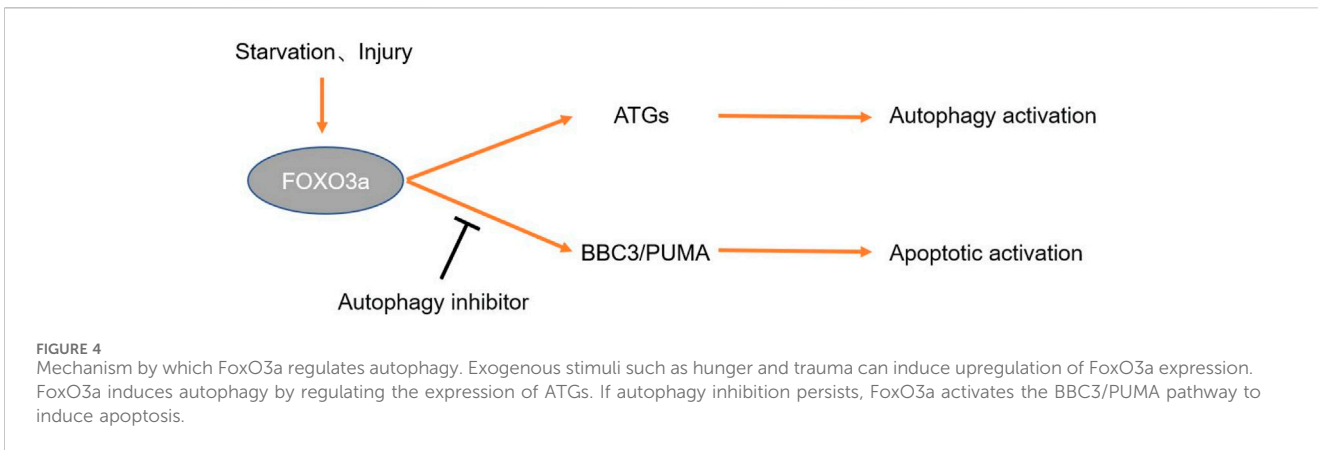
When osteoblasts are injured by oxidative stress, the increase in intracellular ROS induces the activation of FoxO3. Activated FoxO3 induces the expression of a variety of ATGs, thereby promoting osteoblast autophagy to remove organelles damaged by oxidative stress and reducing the osteoblast apoptosis rate by recycling energy-related components and correcting misaligned structures. FoxO3 knockout inhibits the self-purification of cells. However, when oxidative stress damage induced by excessive ROS levels exceeds the phagocytosis induced by Foxo activation, apoptosis is unabated. Oxidative stress injury induced by wear particles has a significant influence in the pathogenesis of PPOL. Therefore, treatment targeting FoxO3 to activate autophagy may increase treatment effectiveness and improve the prognosis of PPOL.

3.4 Beclin1

Beclin1 can form complexes with different proteins, which have different regulatory effects on autophagy. Some studies have shown that Beclin1 forms complexes with class III PI3Ks (hVps34, hVps15) (Hurley and Young, 2017). The Beclin1-hVps34-hVps15 core complex regulates the process of autophagy by forming distinct complexes with Atg14L, UVRAG and Rubicon. The Beclin1–hVps34–hVps15-Atg14L complex facilitates autophagosome nucleation, and the Beclin1–hVps34–hVps15-UVRAG complex facilitates autophagosome maturation. In addition, the Beclin1–hVps34–hVps15-Rubicon complex inhibits autophagy (Xu and Qin, 2019). The binding of Beclin1 and Bcl-2 also regulates autophagy. The combination of Bcl-2/Bcl-xl and Beclin1 inhibits autophagy. Bcl-2/Bcl-xl can bind to

Beclin1 in the BH3 domain under physiological conditions. The dynamic binding of the bcl2-Beclin1 complex can regulate autophagy within a normal level (Kang et al., 2011). The deletion of Beclin1 reduces autophagic flux until it reaches an abnormally low level and leads to cell death finally. In contrast, the loss of Bcl-2 induces overactive autophagy, which may also lead to cell death. c-Jun N-terminal protein kinase 1 (JNK1) phosphorylates Bcl-2, causing the dissociation of the Bcl 2–Beclin1 complex and inducing autophagy (Wei et al., 2008). Overexpression of death-inducing kinase (DAPK) phosphorylates the BH3 domain of Beclin1 and promotes the dissociation of Beclin1 from Bcl-xl, thereby inducing autophagy (Levin-Salomon et al., 2014). ROCK1 can also phosphorylate T119 in Beclin1, resulting in the dissociation of Beclin1 from Bcl-2. The mice showed autophagy inhibition after ROCK1 knockout (Gurkar et al., 2013). Mst1 induces phosphorylation at Thr108 of Beclin1 under stress conditions. On the contrary, Phosphorylation at Thr108 promotes the binding of Beclin1 to Bcl-2/Bcl-xl and stabilizes the Beclin1/Bcl-2 homodimer. The stabilized dimer inhibits the PI3K kinase activity of the Atg14 L–Beclin1–Vps34 complex and ultimately inhibits autophagy (Maejima et al., 2013). Figure 5 shows the composition of the Beclin1 complex and the regulatory mechanism it mediates in conjunction with Bcl-2.

Beclin1 has been identified as an autophagic marker gene because of its key role in autophagy, as well as in autophagy of osteoblasts. PTH1-34 enhances the autophagic activity of osteoblasts by regulating the expression of Beclin1, thereby increasing the proliferation and mineralization of osteoblasts and reducing bone loss (Wu et al., 2021). Many drugs currently used to regulate autophagy in osteoblasts lead to changes in Beclin1 expression. Studies have shown that the expression of Beclin1 in the osteoblasts of osteoporotic rats treated with high-dose resveratrol was increased. Resveratrol treatment also increased the expression of

FIGURE 4
Mechanism by which FoxO3a regulates autophagy. Exogenous stimuli such as hunger and trauma can induce upregulation of FoxO3a expression. FoxO3a induces autophagy by regulating the expression of ATGs. If autophagy inhibition persists, FoxO3a activates the BBC3/PUMA pathway to induce apoptosis.

FIGURE 5
Mechanism by which Beclin1 regulates autophagy. The Beclin1-hVps34-hVps15 core complex promotes autophagy by binding to Atg14L and UVRAG. The binding of the complex to Rubicon decreases autophagic flux. The combination of Beclin1 and Bcl-2 inhibits autophagy; the dissociation of Beclin1 from Bcl-2 promotes autophagy. hVps34: Vacuolar sorting protein VPS34, hVps15: Vacuolar sorting protein VPS15, Atg14 L: Autophagy-related protein 14 L, UVRAG: UV radiation resistance-associated gene protein, Rubicon: RUBCN protein, Bcl-2: B-cell leukemia/lymphoma-2 protein.
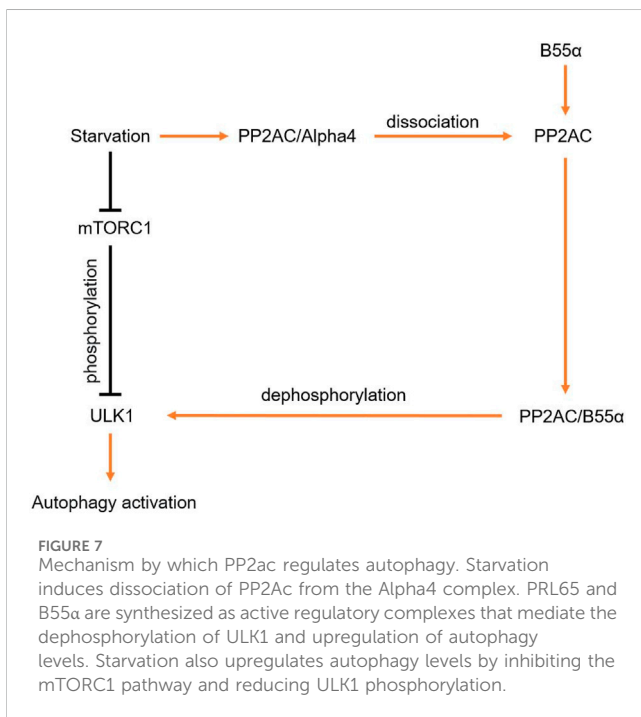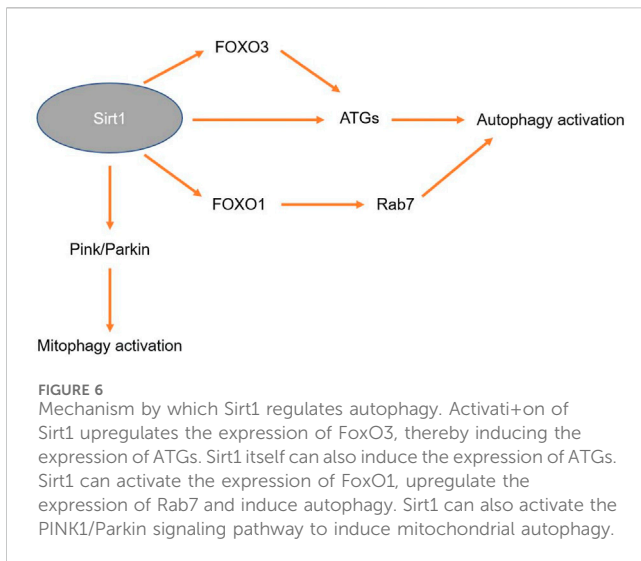
Beclin1 mRNA in dexamethasone (DEX)-treated osteoblasts (Yang et al., 2019).

In summary, the dynamic balance mediated by Beclin1 and Bcl-2 binding is important in maintaining autophagy homeostasis under physiological conditions. The accelerated autophagy induced by wear particles may be ameliorated by regulating Beclin1 and Bcl-2 phosphorylation levels. Beclin1 is also a key component in autophagy maturation and nucleation processes. Therefore, regulating autophagy in osteoblasts by interfering with Beclin1 expression may be important for the treatment of osteolysis near a prosthesis.

3.5 SIRT1

The sirtuin gene family is a highly conserved subfamily of genes that are also class III histone deacetylases (Brachmann et al., 1995).

Sirt1 is mainly expressed in the nucleus of most cell types. Sirt1 is involved in many cellular processes, such as cell senescence, endocrine signal transduction, balanced glucose levels, aging and longevity (Tang, 2016). The regulation of macroautophagy by Sirt1 has been extensively studied. Although Sirt1 is not an important part of the autophagy mechanism, in some cases, the activation of Sirt1 is an important step in activating autophagy (Wang et al., 2021). Sirt1 is able to deacetylate key components of the autophagy activating network (such as the products of the ATGs Atg5, Atg7 and Atg8). Sirt1 also induces key autophagy genes by activating members of the FoxO transcription factor family (Ng and Tang, 2013). Hariharan et al. have shown that Sirt1 deacetylation of FoxO1 is needed for hunger-induced cardiac autophagy and that FoxO1 enhances autophagy flux by activating Rab7 (Hariharan et al., 2010). The upregulation of Sirt1 alleviates MN-induced microglial autophagy dysfunction by regulating FoxO3 deacetylation. The activation of

FIGURE 6
Mechanism by which Sirt1 regulates autophagy. Activati+on of Sirt1 upregulates the expression of FoxO3, thereby inducing the expression of ATGs. Sirt1 itself can also induce the expression of ATGs. Sirt1 can activate the expression of FoxO1, upregulate the expression of Rab7 and induce autophagy. Sirt1 can also activate the PINK1/Parkin signaling pathway to induce mitochondrial autophagy.

FIGURE 7
Mechanism by which PP2ac regulates autophagy. Starvation induces dissociation of PP2Ac from the Alpha4 complex. PRL65 and B55α are synthesized as active regulatory complexes that mediate the dephosphorylation of ULK1 and upregulation of autophagy levels. Starvation also upregulates autophagy levels by inhibiting the mTORC1 pathway and reducing ULK1 phosphorylation.

FoxO3 to LC3B promoter depends on the deacetylation of FoxO3 by Sirt1 (Yan et al., 2023). Therefore, Sirt1 can enhance autophagy by mediating the deacetylation of the FoxO family. The bone mass of Sirt1-knockout mice was reported to be decreased, and the proportion of osteoblasts and osteoclasts was unbalanced. Therefore, Sirt1 has been confirmed to be a direct osteoblast-regulating factor in many studies. The bone formation ability of Sirt1-knockout mice was weakened, which proved a role for Sirt1 in new bone formation. In addition to bone loss caused by Sirt1 deficiency, the tibias of anorexic mice showed increased levels of bone marrow fat (Louvet et al., 2020). The upregulation of Sirt1 induced by seventeen β-estradiol (17β-E2) promoted autophagy through the AMPK/mTOR pathway and inhibited apoptosis by activating FoxO3a in osteoblasts (Aquilano et al., 2013).

As an important regulatory factors of cellular defenses against oxidative stress and cell survival, Sirt1 maintains cell activity by activating autophagy. Sirt1 interferes with autophagy mainly by regulating key pathways in the autophagy network, which is closely related to its role in clearing senile cells, recycling energy-related molecules and inhibiting apoptosis. In addition, Sirt1 may exert an important effect on mitochondrial function. Sirt1 is activated mainly in cells with damaged mitochondria and participates in the regulation of mitophagy. Sirt1 plays a protective role by clearing damaged mitochondria and recycling energy-related molecules. Mitochondrial autophagy is a process by which cells selectively remove damaged mitochondria. Selective removal of damaged mitochondria prevents excessive accumulation of ROS in cells, which is essential for the functional integrity and cell survival of the entire mitochondrial network. Some studies have shown that Sirt1 can mediate PINK1/Parkin-dependent mitochondrial autophagy. Quercetin can enhance mitochondrial autophagy by activating Sirt1-PINK1/Parkin pathways in tubular epithelial cells *in vivo* and *in vitro*, thereby effectively alleviating cellular senescence in tubular epithelial cells and renal interstitial fibrosis (Liu et al., 2020).

Therefore, Sirt1 may play more roles than currently recognized, and therefore, continued research on Sirt1 regulation of autophagy in osteoblasts is both a reasonable and necessary endeavor. Similar to FoxO3, Sirt1 is a key factor in cellular defenses against oxidative stress damage, and the activation of Sirt1 enhances FoxO3 clearance of ROS. The synergistic regulatory effects of Sirt1 and FoxO3 on autophagy and oxidative stress in osteolysis near prostheses need to be further studied. The regulatory effect of Sirt1 on autophagy is briefly shown in Figure 6.

3.6 P62

P62 (sqstm1) is a ubiquitin-binding protein, which is mainly involved in physiological processes such as signal transduction, oxidative stress and autophagy (Komatsu et al., 2007). P62 is a multifunctional protein composed of a variety of different interacting domains (Lin et al., 2013). Through its C-terminal UBA domain or LIR domain, P62 transports ubiquitin to lysosomes for degradation (Myeku and Figueiredo-Pereira, 2011). During autophagy, the degradation of autophagosome cargo in lysosomes leads to a decrease in sqstm1 levels. In contrast, autophagy inhibitor proteins stabilize sqstm1 levels (Katsuragi et al., 2015). P62 functions as an adapter by binding ubiquitinated protein aggregates and trafficking them to lysosomes. The transcription of P62 is mediated by various signaling pathways, such as the oxidative stress response-related (Nrf2), Ras/MAPK, and JNK/c-Jun pathways (Puissant et al., 2012). It is reported that autophagy in osteoblast can be mediated by regulating P62 function. Cannabinoid receptor type 2 (CNR2) induces osteogenic differentiation by activating P62-mediated autophagy (Xu et al., 2020). In rats with type 2 diabetes (T2DM), exercise downregulated long-chain noncoding rnameg3 (meg3) and activated P62-mediated autophagy to promote bone formation (Chen X. et al., 2021). P62 participates in the regulation of the ubiquitin–proteasome system (UPS) through the action of the KEAP1-NrF2 pathway (Liu W. J. et al., 2016). P62 is the

integration center of various cellular functions, including the formation of autophagosomes and the transfer of ubiquitin proteins to lysosomes. P62 is a multidomain protein with multiple domain. The role of some interaction domains in P62 is still poorly understood, thus P62 deserves further study.

3.7 PP2Ac

Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase that regulates phosphorylation in the body (Dzulko et al., 2020). As a trimer, PP2A is composed of catalytic subunit C, structural subunit A and regulatory subunit B. Its function depends mainly on the regulation of catalytic subunit C (Mazhar et al., 2020). On this basis, the diversity of PP2A holoenzyme compositions leads to a variety of possibilities for its regulation in cells. The literature confirms that PP2A has significant influence in the incidence of tumors, respiratory diseases and nervous system diseases. The regulation of PP2Ac methylation levels attenuates MN-induced abnormal autophagy in MN2a cells through the action of the mTORC1/ULK1 signaling pathway (Xu et al., 2021).

ULK1 is a serine/threonine kinase upstream of the autophagy core mechanism, which is a direct substrate for mTORC1. The reversible phosphorylation of ULK1 is a central signaling mechanism that regulates famine-induced autophagy. When the amino acid level is reduced, the activity of mTORC1 is inhibited, and ULK1 is activated simultaneously, thus upregulating the autophagy level (Jung et al., 2009). Wong et al. found that starvation induced autophagy faster and more strongly than mTORC1 inhibitors. This is because starvation can not only block mTORC1 activity but also activate PP2Ac to dephosphorylate ULK1 (Xu et al., 2021). Under physiological conditions, PP2Ac binds to Alpha4 as a complex, which has no regulatory effect on ULK1. Starvation can induce the release of PP2Ac from an inactive complex containing Alpha4 to synthesize an active regulatory complex with PRL65 and B55α, thereby mediating the dephosphorylation of ULK1 (Wong et al., 2015). The regulatory effect of PP2Ac on autophagy is briefly shown in Figure 7

The expression of PP2Ac was significantly increased in macrophages infected with *Mycobacterium bovis*. Inhibition of PP2Ac by nilotinib conferred protection through autophagy onto macrophages by activating the AMPK signaling pathway, thus increasing the antimicrobial function and survivability of the cells (Hussain et al., 2019). Taurine blocked mitophagy by inhibiting PP2Ac methylation, thereby reducing excessive polarization of macrophages into the proinflammatory type. VDAC1 and PINK1 are mediators of PP2Ac methylation-induced mitophagy and are needed to maintain PP2Ac hypermethylation (Meng et al., 2021). In decades, many scholars have proved that PP2A is related to orthopedic diseases. Okamura H et al. found that a reduction in PP2A levels promoted osteoblast differentiation by regulating Osterix and other bone-related transcription factors (Okamura et al., 2017). PP2ac can coordinate with the mTOR/ULK1 pathway to induce rapid autophagy activation during cell starvation. In addition, PP2ac is also closely related to a variety of autophagy signaling pathways, such as PINK1/Parkin and AMPK. It also plays an important role in bone metabolic diseases. PP2ac deserves further study as a potential regulator of osteoblast function.

4 The mitophagy signaling pathway in osteoblasts

The object of autophagy can be a large number of non-selective proteins or a single cell component that is highly regulated, depending on the inducible factor. As a selective autophagy, mitophagy has been extensively studied. Mitophagy regulates the quality and quantity of mitochondria by selectively degrading mitochondria. Specific mitochondrial outer membrane receptors or ubiquitin molecules coupled to mitochondrial surface proteins mediate the formation of the autophagosome around the mitochondria (Onishi et al., 2021). Mitochondrial autophagy mediates variety important processes such as cell growth and development, apoptosis and inflammation. Mitophagy also plays a protective role against cell proliferation and differentiation in its role as an "intracellular steward" (Lou et al., 2020).

4.1 PINK1/PARKIN

Parkin is composed of 465 amino acids and is encoded by the second largest human gene after antinutrophin. In 1998, Parkin was identified as the pathogenic gene underlying autosomal recessive juvenile Parkinson's disease (AR-JP) (Pickrell and Youle, 2015). PINK1 is another pathogenic gene associated with early-onset Parkinson's disease, including AR-JP (Singleton et al., 2013). PINK1 and Parkin exhibit different molecular functions: one is a protein kinase, the other is a ubiquitin ligase, but they have the same pathogenic effect. PINK1/Parkin, as a classical mitochondrial self-regulation pathway, has been widely studied by scholars (Nardin et al., 2016). PINK1, as a surveillance molecule, constantly monitors the health of mitochondria under physiological conditions and responds quickly when mitochondria are damaged. In healthy mitochondria, PINK1 is rapidly degraded by the UPS during its passage through mitochondrial membranes. In contrast, damaged mitochondria cause PINK1 accumulation due to the loss of membrane potential. This leads to rapid recruitment and activation of Parkin and subsequent initiation of mitophagy (Nguyen et al., 2016). Under physiological conditions, Parkin is maintained in a "closed" state through multiple intramolecular interactions, making its inhibitory state the basal condition. PINK1 activates Parkin by directly phosphorylating Ser65 in the Parkin Ubl domain (Bingol and Sheng, 2016). Mutations in both Parkin and PINK led to significant inhibition of mitochondrial protein renewal and mitophagy. The accumulation of damaged mitochondria is the main cause of Parkinson's disease (Wang et al., 2016). In decades, the PINK1/Parkin pathway has been proved to have great influence in osteoblast function. Parkin activates the β-catenin signaling pathway to enhance autophagy and promote the differentiation of BMSCs into osteoblasts. In contrast, self-regulation of Parkin expression leads to accelerated osteoblastic differentiation, causing steatosis of MSCs and reduced expression of type I collagen (Zhang W. et al., 2020). VK2 reversed the inhibition of dexamethasone-induced autophagy by upregulating the expression of Parkin and other autophagy and mitochondrial markers, thereby increasing osteoblast function (Chen et al., 2020). These findings suggest that in-depth study of the PINK1/

Parkin activation mechanism may lead to a better understanding and new ideas for autophagy-related cytoprotection.

5 Discussion

To date, the pathological mechanism underlying PPOL has not been fully clarified. The function and metabolic changes in many cells of bone tissue are involved. Osteoblasts, derived from BMSCs, are important sources of bone cells that represent the final differentiation state of osteoblast cell lines. Therefore, changes in the function and activity of osteoblasts have an indispensable influence on the occurrence and development of PPOL. Autophagy, as a self-protective and self-purification process, plays a substantial role in maintaining the function and activity of osteoblasts. A large amount of credible evidence suggests that autophagy is involved in the development and differentiation of osteoblasts and the maintenance of bone homeostasis. Activation of autophagy in response to environmental stress or foreign stimuli enhances osteoblast activity and inhibits osteoblast apoptosis. Activation of autophagy is indispensable in the early stages of BMSC differentiation into osteoblasts. Moreover, the activation of autophagy alleviates the oxidative stress damage induced by Ti particles and increases the antioxidative capacity of osteoblasts. Since autophagosomes are vehicles for secreting apatite crystals into the extracellular space, autophagy is identified as necessary for the initiation of osteoblast mineralization. Impaired autophagy causes osteoblast failure to clear damaged organelles or remain viable in a nutrient-deficient state, which leads to osteoblast apoptosis and disrupts the bone-remodeling balance. In contrast, the overactivation of autophagy leads to irreversible osteoblast self-degradation, which causes dysregulated bone homeostasis. Therefore, autophagy regulatory mechanisms have been proven to increase the activity and function of osteoblasts and thus play important roles in metabolic diseases of bone. In this review, we summarized and discussed several signaling pathways that have been shown to regulate autophagy in osteoblasts under physiological conditions and during pathological changes in bone metabolism. AMPK, mTOR and ULK1 regulate each other to maintain autophagy stability and prevent excessive autophagy activation. Crosstalk between the NF-κB signaling pathway and autophagy pathway components is complex. Activation of NF-κB signaling may exert opposite effects on autophagy in different cells or diseased tissue. NF-κB mainly plays a role in affecting downstream autophagy genes, such as Binp3, Beclin1, and mTOR. FoxO3 mainly mediates autophagy initiated by nutrient deficiency. FoxO3 is activated during cell starvation to mediate autophagy. However, continuous inhibition of autophagy can induce FOXO3 to initiate apoptosis. Beclin1 plays a significant role in the generation and regulation of autophagy, as it is a key component of autophagosome formation. Beclin1 induces autophagosome nucleation and maturation by forming complexes with different molecules. Moreover, its dynamic binding with Bcl-2 is crucial for the stability of autophagic flux. Sirt1 affects autophagy mainly through its regulation of key components in the autophagy induction network; these components include Atg5, Atg7 and Atg8. Sirt1 also induces the expression of autophagy pathway components by activating FoxO family members. However, the

autophagic gene network is constructed by multiple signaling pathways, and multiple signaling pathway components engage in crosstalk. Unfortunately, research on autophagy mechanisms to date has been insufficient, and more in-depth research is still needed. The autophagy signaling pathway in osteoblasts reviewed in this paper is only part of the autophagic gene network, and the activation and action modes of each signaling pathway may not be limited to those discussed herein. Future research should focus on the crosstalk among different autophagic signaling pathway components so that we can better understand the complex autophagic gene network.

Most studies have focused on the effect of autophagy inhibitors on osteolysis. 3-MA and LY294002 have been shown to inhibit osteoclast generation and reduce the expression of pro-inflammatory factors *in vitro*, thus playing a certain therapeutic role in PPOL (Chen W. et al., 2021). 3-MA can also reduce CoCrMo granulose-induced osteoblast apoptosis by inhibiting autophagy (Wang et al., 2015). However, due to the complexity of the autophagy network, the role of autophagy in PPOL has been controversial and no clear conclusions have been drawn so far. Inhibition or activation of autophagy alone may be difficult to achieve a therapeutic effect because autophagy does not work consistently in different cells. And autophagy also plays different roles at different times in the same cell. For example, autophagy is an essential condition in the mineralization process of osteoblasts, but excessive autophagy can mediate apoptosis of osteoblasts. Therefore, enhancing the precision of autophagy control or maintaining appropriate autophagy strength may be helpful for future PPOL treatment. Due to the single mechanism and effect of most drugs, it is difficult to accurately regulate the intensity of autophagy. And the long-term safety of some drugs remains to be determined. Further research on the regulation of key genes in autophagy may be helpful. How to regulate key genes in the autophagy process of specific cells, instead of interfering with the overall level of autophagy, is our next research direction.

PPOL, a common and difficult-to-treat complication of joint replacement surgery, affects the majority of patients. Improving the function and activity of osteoblasts has great significance in improving the prognosis of PPOL. Autophagy regulation has been proven to reduce the osteoblast apoptosis rate and increase osteoblast differentiation and mineralization. In summary, we hope to provide new research directions and therapeutic targets for the treatment of PPOL and improve prognosis by summarizing the latest information on autophagy signaling pathways. However, the autophagy gene network is composed of multiple signaling pathways, and crosstalk among these signaling pathways is complex. To date, our understanding of the mechanism of autophagy is still insufficient. The autophagy signaling pathways in osteoblasts reviewed in this paper constitute only a portion of the autophagy gene network, and the activation and action modes of each signaling pathway may involve more mechanisms than are described herein. In future studies, we will focus on analyzing the crosstalk between various autophagy signaling pathway components. Through drug intervention or gene-targeted regulation, the autophagic flux in osteoblasts in the wear particle-influenced microenvironment has been shown to be regulated within a stable range. We should not only pay full attention to the positive effects of autophagy on osteoblasts but also prevent the damage caused by the excessive activation of autophagy, which is the focus of our subsequent research.

Author contributions

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Conflict of interest

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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