Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 2024 25(3):197-211
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Review

https://doi.org/10.1631/jzus.B2300402

Advances in the study of mitophagy in osteoarthritis

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Abstract: Osteoarthritis (OA), characterized by cartilage degeneration, synovial inflammation, and subchondral bone remodeling, is among the most common musculoskeletal disorders globally in people over 60 years of age. The initiation and progression of OA involves the abnormal metabolism of chondrocytes as an important pathogenic process. Cartilage degeneration features mitochondrial dysfunction as one of the important causative factors of abnormal chondrocyte metabolism. Therefore, maintaining mitochondrial homeostasis is an important strategy to mitigate OA. Mitophagy is a vital process for autophagosomes to target, engulf, and remove damaged and dysfunctional mitochondria, thereby maintaining mitochondrial homeostasis. Cumulative studies have revealed a strong association between mitophagy and OA, suggesting that the regulation of mitophagy may be a novel therapeutic direction for OA. By reviewing the literature on mitophagy and OA published in recent years, this paper elaborates the potential mechanism of mitophagy regulating OA, thus providing a theoretical basis for studies related to mitophagy to develop new treatment options for OA.

Key words: Mitophagy; Osteoarthritis; Chondrocyte; Mitochondria; Apoptosis

1 Introduction

Osteoarthritis (OA) is a chronic joint disease characterized by the degeneration of articular cartilage, subchondral bone remodeling, osteophyte formation, synovial inflammation, and extensive vascularization (Glyn-Jones et al., 2015). According to the World Health Organization (WHO), OA, as one of the most common musculoskeletal disorders, has a prevalence of 50% in people over the age of 50 years and 80% in people over the age of 55 years, affecting more than 650 million people worldwide (Cui et al., 2020). Aging, obesity, metabolic inflammation, joint trauma, and deformity are all risk factors for OA initiation (Russell et al., 2013; Davis et al., 2017; Kim et al., 2018; Vina and Kwoh, 2018). Articular cartilage is made up of chondrocytes and the extracellular matrix (ECM). As the only cell type within articular cartilage, chondrocytes secrete a variety of collagen fibers (types II, IX, and XI collagen fibers), proteoglycan (PG), and other non-collagenous matrix proteins that function to maintain cellular activity and cartilage homeostasis (Paulsson and Heinegård, 1979; Eyre, 2002; Kiani et al., 2002; Schulz and Bader, 2007). These matrix molecules can induce a significant level of apoptosis in chondrocytes when they are broken down in large numbers (Kim et al., 2001), which in turn leads to a reduction in their ability to secrete cartilage matrix, creating a vicious cycle that causes the progressive destruction and breakdown of articular cartilage (Kop'eva et al., 1986). Although chondrocytes represent only 1% of the total articular cartilage volume, chondrocyte activity and their ability to secrete ECM are essential for normal cartilage function (Charlier et al., 2019). The anabolism and catabolism of healthy chondrocytes are in dynamic stability, which maintains cartilage homeostasis. In contrast, OA chondrocytes exhibit high levels of catabolism, low levels of anabolism, reduced cellular activity, and mitochondrial dysfunction (Sun et al., 2021; Fernández-Moreno

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Received June 11, 2023; Revision accepted Aug. 21, 2023;
Crosschecked Feb. 22, 2024

et al., 2022; Liu et al., 2022). Mitochondria are the sites of intracellular tricarboxylic acid cycle and oxidative phosphorylation, the power station of the cell (Friedman and Nunnari, 2014), and the main source of reactive oxidative species (ROS) generation (Tahrir et al., 2019). Accumulating evidence suggests that OA is closely associated with mitochondrial dysfunction. Abnormalities in the mitochondrial electron transport chain in OA chondrocytes compared to healthy chondrocytes result in the abnormal production of ROS in the mitochondria and mutations in mitochondrial DNA (mtDNA). ROS can be involved in the regulation of various physiological activities, including cell survival and inflammatory responses (Scherz-Shouval et al., 2007; Bulua et al., 2011; Edgar et al., 2012). However, the accumulation of ROS leads to an imbalance between oxidative and antioxidant effects to activate the oxidative stress response, which is an important factor in aging and joint disease (Jones, 2015; Arra et al., 2020). Abnormally high levels of ROS in chondrocytes can promote the production of matrix metalloproteinases (MMPs) to degrade the cartilage ECM and activate pro-apoptotic p38 signaling, leading to chondrocyte apoptosis (Reed et al., 2014; Collins et al., 2016). High levels of ROS also act on mitochondria, compromising mtDNA integrity and facilitating the escape of mtDNA to the cytoplasm through the open permeability transition pore in the outer mitochondrial membrane (OMM). Subsequently, mtDNA entering the cytoplasm binds to nucleotide-binding oligomerization domain (NOD)-like receptor thermal protein domain-associated protein 3 (NLRP3) and cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS), which jointly trigger the inflammatory response (Xian et al., 2022). Thus, mitochondrial dysfunction leads to abnormal ROS production and mtDNA escape, exacerbating the deterioration and progression of OA (Blanco et al., 2011; Wu LH et al., 2014; Ansari et al., 2020).

Fortunately, organisms have derived a mitochondrial self-functioning security mechanism, mitochondrial quality control (MQC), that maintains the mitochondrial number and ensures mitochondrial structural and functional integrity. When cells are under mild stress, they can maintain the functional integrity of mitochondria by removing damaged mitochondria through mitochondrial fusion, division, and mitophagy. Under higher stress levels, cells can also initiate mitochondrial apoptosis to ensure the structural and functional integrity of mitochondria (Dawson and Dawson, 2017; Higuchi-Sanabria et al., 2018). Mitophagy, as MQC at the level of mitochondrial organelles, is a way for autophagosomes to target and engulf damaged or dysfunctional mitochondria to remove them, thereby maintaining mitochondrial homeostasis (Duan and Fang, 2016). The effect of mitophagy on cellular activity depends on the level of mitophagy. Appropriate mitophagy is a self-protective mechanism that promotes cell survival under stress; however, excessive mitophagy can also cause mitophagy-associated apoptosis due to the disruption of intracellular energy supply caused by mitochondrial deprivation (Xu et al., 2019). Therefore, a healthy level of mitophagy is essential to maintain mitochondrial homeostasis and ensure cell survival. Currently, it is considered that mitophagy is closely associated with the initiation and development of OA. On the one hand, mitophagy can inhibit OA initiation by removing dysfunctional mitochondria, reducing mitochondrial ROS production, and inhibiting chondrocyte apoptosis (Ansari et al., 2018; Bernardini et al., 2019). On the other hand, it has been found that the activation of mitophagy also triggers the caspase/cleaved caspase-3 apoptotic cascade, which instead exacerbates apoptosis (Lu et al., 2021). At present, the study of the role of mitophagy in OA is still in the early stage, and the potential mechanisms by which mitophagy regulates OA are still unclear. In this paper, by reviewing the research literature related to mitophagy and OA, the specific mechanism of mitophagy regulating chondrocyte homeostasis and some current problems are summarized, with the aim to reveal the specific role of chondrocyte mitophagy in OA and provide new potential targets for OA treatment.

2 Overview of autophagy and mitophagy

Autophagy is the normal dynamic life process in which cells use lysosomes to degrade and selectively remove their own damaged, senescent, or excess biomolecules and organelles while releasing free small molecules for cellular recycling. Depending on the mode of intracellular substance delivery to the lysosome, autophagy is subdivided into macroautophagy, microautophagy, and chaperone-mediated autophagy. Autophagy was originally described as a non-selective

holistic process induced by starvation. It occurs in four stages as follows. (1) Initiation of autophagy. In response to stimuli such as starvation or oxidative stress, the UNC-51-like kinase 1 (ULK1) forms the ULK1 complex with multiple autophagy-related proteins such as autophagy-related gene 13 (ATG13), ATG101, and focal adhesion kinase family interacting protein of 200 kD (FIP200). ATG13 can anchor the ULK1 complex to the autophagy precursor (pre-autophagosomal structure (PAS)). When multiple ATG proteins co-aggregate on the PAS scaffold, autophagy is initiated (Suzuki et al., 2001; Kawamata et al., 2008; Yamamoto et al., 2016). (2) Autophagosome assembly and formation. The ULK1 complex further recruits the phosphoinositide 3-kinase (PI3K) complex, ATG9A system, ATG12-coupled system, and ATG8 family proteins (microtubule-associated protein 1 light chain 3 (LC3)-coupled system and γ-aminobutyric acid type A receptor-associated protein (GABARAP) system) to the PAS scaffold to promote vesicle nucleation, extension, and closure. Among them, the lipidation of LC3/GABARAP by phosphatidylethanolamine (PE), a key molecular event for autophagosome formation, is manifested by the nucleation of autophagosomes (forming a bowl-shaped structure called phagophore). Subsequently, the phagophore continues to elongate and extend to the sides, gradually enveloping the components (organelles, aggregated proteins, etc.) within the envelope. Finally, the phagophore closes to form a closed spherical bilayer membrane structure known as mature autophagosomes (Cheong et al., 2008; Suzuki et al., 2013). (3) Autophagosome and lysosome fusion. Autophagosomes are translocated to the perinuclear region where lysosomes are located and undergo autophagosome-lysosome fusion (Kimura et al., 2008). (4) Degradation and recycling of autophagosomal contents. Lysosomes degrade the endosomal membrane and autophagosome-engulfed substances into amino acids or polypeptides for cellular reuse.

Current research has revealed that the turnover of damaged organelles, the clearance of protein aggregates, and the elimination of intracellular pathogens are highly selective and tightly regulated processes, also referred to as selective autophagy. The identified selective autophagy processes include aggrephagy, mitophagy, pexophagy, ribophagy, reticulophagy, and xenophagy (Stolz et al., 2014). Selective autophagy is usually achieved via autophagy-associated receptors containing LC3-interaction regions (LIRs) or GABARAP interaction motifs (GIMs) that bind to LC3/GABARAP family members on phagocytic vesicles and promote selective autophagy. Non-selective autophagy plays an important role in cell starvation by providing amino acids required for cell nutrition through the digestion of cell structures to ensure cell survival, while selective autophagy is mainly used to protect intracellular structures and sweep the cell of damaged organelles and pathogens to maintain cellular homeostasis (Anding and Baehrecke, 2017). Selective autophagy can target damaged organelles and aggregated proteins for efficient degradation, which is more specific than general autophagy, thus better maintaining organelle homeostasis.

Mitophagy, a form of selective autophagy, is a specific form of autophagy in which autophagosomes selectively target damaged and depolarized mitochondria for degradation (Williams et al., 2017). Mitochondria serve as key organelles providing energy for various cellular life activities. Stress stimuli can lead to mitochondrial damage or dysfunction, which causes a decrease in mitochondrial membrane potential (MMP), that is, mitochondrial membrane depolarization. Normal MMP is necessary to maintain mitochondria for oxidative phosphorylation and adenosine triphosphate (ATP) production. Mitochondrial membrane depolarization activates mitophagy to remove damaged mitochondria. Mitophagy is a normal life activity in the organism that performs organelle-level MQC through targeted engulfing of damaged mitochondria to maintain normal mitochondrial numbers and ensure mitochondrial structural and functional integrity. Accumulating evidence suggests that abnormal mitophagy is associated with multiple pathological changes or disease progression, such as aging (Fivenson et al., 2017), cardiovascular disease (Pedro et al., 2017), and Alzheimer's disease (Kerr et al., 2017). Recent studies have shown that abnormal mitophagy also plays a key role in musculoskeletal disorders, especially OA (Wang S et al., 2020; Sun et al., 2021).

3 Mitophagy and osteoarthritis

Mitophagy is closely associated with the initiation and development of OA. Because of the lack of blood supply to the articular cartilage (Hunter, 1995), oxygen and nutrients are mainly carried by synovial

fluid for the exchange of substances with the subchondral bone (Imhof et al., 2000). The nutritional deprivation combined with the resting state and low proliferation rate of chondrocytes results in the need for chondrocytes to maintain cell survival and normal function through higher levels of autophagy (Cuervo et al., 2005; Glick et al., 2010; Lu et al., 2021). Mitochondria are the power station of the cell and the main source of ROS generation. Mitochondrial dysfunction and associated oxidative stress are highly related with chondrocyte activity (Coryell et al., 2021). Dysfunctional mitochondria will result in abnormal production of ROS, and the accumulation of ROS in turn can lead to chondrocyte apoptosis and cartilage matrix degradation by activating oxidative stress, ultimately exacerbating OA. It is widely believed that mitophagy is a cellular self-protection mechanism that can reduce oxidative stress within chondrocytes by removing dysfunctional mitochondria, thereby inhibiting chondrocyte apoptosis and ultimately alleviating OA progression (Tang et al., 2017; Ansari et al., 2018; Bernardini et al., 2019). Promoting mitophagy levels and restoring autophagic flux can partially reverse oxidative stress-mediated mitochondrial dysfunction to avoid chondrocyte apoptosis (D'Adamo et al., 2020). However, it has also been found that the over-activation of mitophagy also triggers the mitochondria-related apoptotic cascade, which instead exacerbates apoptosis and disease progression (Lu et al., 2021). Excessive mitophagy can also cause mitophagy-associated apoptosis due to the disruption of intracellular energy supply caused by mitochondrial deprivation (Xu et al., 2019). Studies have indicated a high expression of mitophagy-related genes in both OA patient-derived cartilage and sodium iodoacetate-induced rat OA cartilage (Shin et al., 2019; Yu et al., 2022). This is similar to chondroptosis, a specific form of cell death that occurs during the progression of OA disease. Chondroptosis not only has apoptotic properties (accumulation of caspase in the cell) but also autophagic characteristics (swelling of the endoplasmic reticulum and Golgi apparatus). The swollen endoplasmic reticulum membrane provides autophagic vesicles for cytoplasmic and organelle digestion, leading to the total elimination of cellular debris (Salucci et al., 2022). Therefore, it has been suggested that chondroptosis is a combination of classical apoptosis and autophagy, which may be an autophagy-related apoptotic response presented by chondrocytes after failure to respond to stress (Almonte-Becerril et al., 2010). Fig. 1 shows two different mitophagy functions under mitochondrial stress and their related outcome. In the normal mitophagy situation, under mitochondria stress, mitophagy is activated to remove the damaged mitochondria and relieve the stress, so that eventually the cells can survive. In the impaired mitophagy situation, impaired mitophagy under mitochondrial stress leads to mitochondria-dependent apoptotic cascade that promotes apoptosis as well as OA progression. Only normal mitophagy can promote cell survival in response to external stimuli, whereas excessive or decreased mitophagy can cause mitophagy-associated apoptosis due to the disruption of intracellular energy supply caused by mitochondrial deprivation.

Existing studies have identified two pathways of mitophagy: Parkin RBR E3 ubiquitin protein ligase (PRKN)-dependent and -independent (as shown in Table 1), both associated with the development of OA (Koyano et al., 2014; Nguyen et al., 2016; Blanco and Rego-Pérez, 2018). These findings contribute to the understanding of the role of mitophagy in OA and suggest that the regulation of mitophagy is a potent target for OA therapy.

3.1 PRKN-dependent pathway

The PINK-dependent pathway, a widely occurring mitophagy pathway, is mediated by phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) and PRKN. The initiation of mitophagy depends on the recognition of damaged and normal mitochondria. In the PINK/PRKN pathway, mitophagy is mainly initiated by the abnormal accumulation of PINK1 in the OMM. PINK1 is a serine/threonine protein kinase mainly located in mitochondria. Under normal conditions, PINK1 will be translocated to the inner mitochondrial membrane (IMM) and cleaved by presenilin-associated rhomboid-like protein (PARL). Cleaved PINK1 is subsequently transported to the cytosol for proteasomal degradation. When the mitochondrial membrane is depolarized, PINK1 cannot enter the IMM. Under the action of OMM translocase, PINK1 accumulates in the OMM and undergoes autophosphorylation at Ser228. Activated PINK1 that accumulates in large amounts in the OMM can phosphorylate ubiquitin, thereby recruiting the E3 ubiquitin enzyme Parkin to mitochondria (Koyano et al., 2014). Subsequently, PRKN is activated by phosphorylated

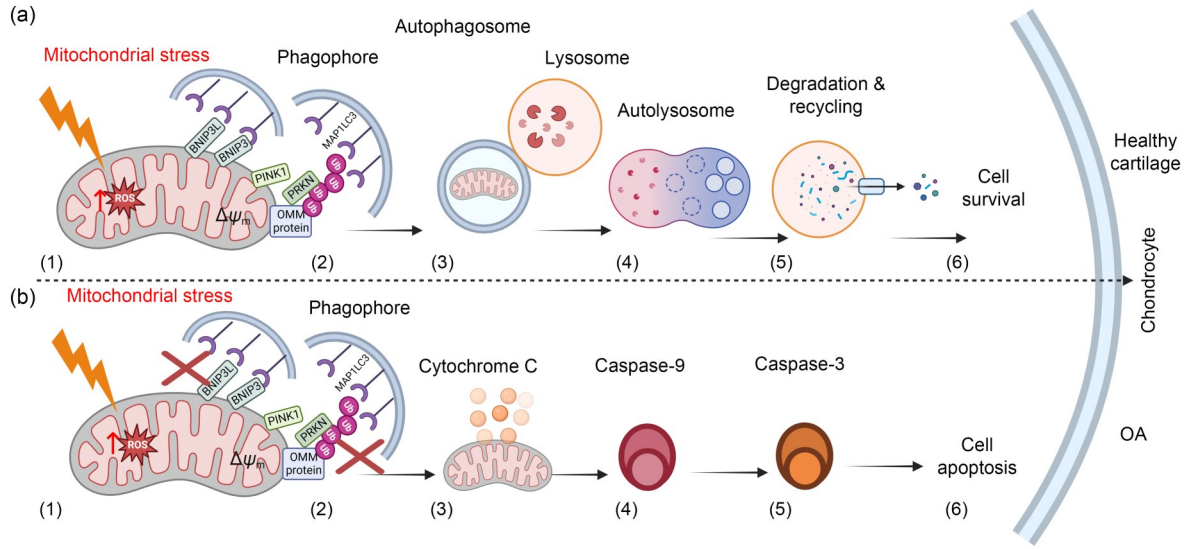

Fig. 1 Mitophagy process and OA progression. (a) Mitophagy is activated to remove damaged mitochondria: (1) Mitochondrial stress occurs, featured by a large accumulation of ROS and membrane depolarization; (2) Mitochondrial membrane depolarization activates mitophagy mediated by PINK1 and BNIP3, forming the bowl-shaped phagophore that gradually envelops damaged mitochondria; (3) The phagophore completely envelops the damaged mitochondria to form a mature autophagosome, which is transported to the perinuclear region where the lysosomes are located; (4) The autophagosome fuses with lysosomes to form an autolysosome; (5) Lysosomes degrade the endosomal membrane and autophagosomal engulfed mitochondria; (6) The damaged mitochondria are removed to relieve the cellular stress and the cell survives. (b) Impaired mitophagy after mitochondrial stress leads to apoptosis: (1) Mitochondrial stress occurs, featuring the large accumulation of ROS and membrane depolarization; (2) The function of mitophagy is impaired; (3) The damaged mitochondria release cytochrome C that initiates the apoptotic cascade; (4, 5) Cytochrome C forms an apoptosome with caspase-9 to summon and activate caspase-3, triggering the apoptotic cascade; (6) The apoptotic cascade eventually causes apoptosis. OA: osteoarthritis; ROS: reactive oxidative species; BNIP3: B-cell lymphoma-2 (BCL-2)/adenovirus E1B 19 kDa-interacting protein 3; BNIP3L: BNIP3-like; PINK1: phosphatase and tensin homolog (PTEN)-induced putative kinase 1; PRKN: Parkin RBR E3 ubiquitin protein ligase; OMM: outer mitochondrial membrane; $\Delta\psi_m$: mitochondrial membrane potential; Ub: ubiquitin; MAP1LC3: microtubule-associated protein 1 light chain 3 (LC3).

ubiquitin and PINK1 to utilize its E3 ubiquitin ligase activity to ubiquitinate the OMM proteins such as voltage-dependent anion-selective channel protein 1 (VDAC1) and mitofusin 1/2 (Mfn1/2) (Sarraf et al., 2013). These mitochondrial membrane proteins contain LIRs that can bind to LC3 and recruit autophagosomes to induce mitophagy (Sarraf et al., 2013). Mitochondria that are subsequently marked by ubiquitination are engulfed by autophagosomes and fused with lysosomes for complete degradation.

In the PINK/PRKN-dependent pathway, PINK1 acts as a detector for mitochondrial damage and a sensing device for mitophagy initiation, while the ubiquitination of mitogen-associated proteins by PRKN (Parkin) is a critical step of mitophagy. The blocked ubiquitination of membrane proteins by deubiquitinating enzymes can significantly inhibit the level of mitophagy (Bingol et al., 2014; Wang et al., 2015). However, PINK1 can also interact with mitochondrial poly(A) polymerase (MTPAP) by recruiting the autophagy receptor protein calcium-binding and coiled-coil domain 2 (CALCOCO2) alone. Then, PINK1 enables the recognition of damaged mitochondria by autophagic vesicles in an ubiquitin-independent manner (Lazarou et al., 2015; Furuya et al., 2018). Another novel model proposed by other teams is that phosphate ubiquitin produced by PINK1 acts as a mitophagy signal that is amplified by Parkin. It was found that PINK1 recruited the autophagy receptors optineurin (OPTN) and nuclear dot protein 52 (NDP52) to mitochondria through its ubiquitin-binding domain to initiate mitophagy in the absence of Parkin, suggesting that PINK1 can mediate mitophagy in a Parkin-independent manner. However, the presence of Parkin can amplify the PINK1-induced signaling pathway and enhance the mitophagy process (Lazarou et al., 2015).

The PINK/PRKN is a key pathway of mitophagy, which is closely related to the development of OA.

Table 1 Mitophagy in osteoarthritis

Potential target	Modeling method	Subject	Mitochondrial function	Function	Reference
Parkin	IL-1β treatment	Human chondrocytes	Promotion	Alleviate OA	Ansari et al., 2018
PINK1	MIA treatment	SW1353 cell	Promotion	Alleviate OA	Huang et al., 2020
PINK1/Parkin	DMM modeling	Human healthy and OA chondrocytes and OA mouse	Promotion	Alleviate OA	Fivenson et al., 2017
PINK1	TBHP and DMM modeling	Senescent chondrocytes and OA mouse	Promotion	Alleviate OA	Jiang et al., 2022
AMPK/PINK1/Parkin	IL-1β and MIA modeling	Chondrocytes and OA rat	Promotion	Alleviate OA	Jin et al., 2022
SIRT3/PINK1/Parkin	DMM modeling	Human OA chondrocytes and OA mouse	Promotion	Alleviate OA	Wang FS et al., 2020
AMPK/Parkin	IL-1β treatment	Chondrocytes	Promotion	Alleviate OA	Maimaitijuma et al., 2020
SIRT3/Parkin	IL-1β treatment	Human OA chondrocytes	Promotion	Alleviate OA	Xin et al., 2022
SIRT3/PINK1/Parkin	IL-1β treatment	Chondrocytes	Promotion	Alleviate OA	Wang CZ et al., 2018
PINK1/Parkin	IL-1β treatment	Rat chondrocytes	Promotion	Alleviate OA	He and He, 2023
Parkin	DMM modeling and IL-1β treatment	Human and rat chondrocytes and OA rat	Promotion	Alleviate OA	Xu et al., 2020
PINK1/Parkin	DMM modeling	Human healthy and OA chondrocytes and OA mouse	Promotion	Alleviate OA	D'Amico et al., 2022
PINK1/Parkin	IL-1β treatment and ACLT modeling	Human healthy and OA chondrocytes and OA rat	Promotion	Alleviate OA	Liu et al., 2023
HIF1α/PINK1/Parkin	Hypoxia stimulation and IL-1β treatment	C28/I2	Promotion	Accelerate OA	Lu et al., 2021
PINK1	MIA modeling	SD rats and human OA chondrocytes	Promotion	Accelerate OA	Shin et al., 2019
PINK1/Parkin	Hulth modeling and LPS treatment	OA rat and chondrocytes	Promotion	Accelerate OA	Yu et al., 2022
AMPK/ULK1/BNIP3	TBHP treatment	Human OA chondrocytes and mouse chondrocytes	Promotion	Alleviate OA	Tang et al., 2017
SIRT1/AMPK/mTOR	Serum-starvation	ATDC5	Promotion	Alleviate OA	Mei et al., 2021
HIF1α/BNIP3	DMM modeling and hypoxia stimulation	Human healthy and OA chondrocytes and OA mouse	Promotion	Alleviate OA	Hu et al., 2020
BNIP3	LPS treatment	ATDC5	Promotion	Alleviate OA	Ma et al., 2020
BNIP3	TBHP treatment	Human OA cartilage and chondrocytes	Promotion	Alleviate OA	Shang et al., 2023
HIF1α/BNIP3	Hypoxia stimulation and IL-1β treatment	C28/I2	Promotion	Accelerate OA	Lu et al., 2021
BNIP3	DMM modeling	Human healthy and OA chondrocytes and OA mouse	Promotion	Accelerate OA	Kim et al., 2021

OA: osteoarthritis; IL-1β: interleukin-1β; PINK1: phosphatase and tensin homolog (PTEN)-induced putative kinase 1; MIA: monoiodoacetate; DMM: destabilization of medial meniscus; TBHP: tert-butyl hydrogen peroxide; AMPK: adenosine monophosphate (AMP)-activated protein kinase; SIRT3: Sirtuin 3; ACLT: anterior cruciate ligament transection; HIF1α: hypoxia-inducible factor 1α; LPS: lipopolysaccharide; ULK1: UNC-51-like kinase 1; BNIP3: B-cell lymphoma-2 (BCL-2)/adenovirus E1B 19 kDa-interacting protein 3; mTOR: mammalian target of rapamycin.

Many studies have encountered problems when chondrocytes were treated with sodium monoiodoacetate (MIA) or interleukin-1β (IL-1β) to simulate the pathological conditions of OA in vitro, including increased levels of ROS, mitochondrial damage, and the decrease of PINK1 and Parkin expression, which seriously affected the level of mitophagy and cell activity (Ansari et al., 2018; Huang et al., 2020). However, Parkin overexpression, activation of PINK1, and addition of zinc, urolithin A, and Baicalin could enhance the level of mitophagy, thereby reversing apoptosis (Ansari et al., 2018; Huang et al., 2020; D'Amico et al., 2022; Jiang

et al., 2022; He and He, 2023). Existing studies have found that a variety of upstream molecules can mediate mitophagy and OA through the PINK/PRKN pathway, including AMP-activated protein kinase (AMPK) and Sirtuin 3 (SIRT3) (Wang FS et al., 2020).

3.1.1 AMPK signaling

AMPK is a serine/threonine protein kinase involved in the regulation of energy homeostasis. Under stress stimuli, such as starvation and hypoxia, the cell energy decreases and the AMP/ATP ratio increases. AMPK is then activated to increase ATP production and reduce ATP consumption to maintain the intracellular energy balance (Hardie, 2014; Ibrahim et al., 2015; Tamrakar et al., 2015). AMPK can promote the autophagy of chondrocytes to play a protective role in cartilage. There is evidence that AMPK activity is reduced in the cartilage of OA model or aged mice, consistent with the altered levels of autophagy (Petursson et al., 2013; Duan et al., 2020). AMPK can promote the overall level of autophagy by inhibiting the mammalian target of rapamycin (mTOR), a key molecule in the autophagy inhibition pathway (Feng et al., 2020). Plant homeodomain finger protein 23 (PHF23) controlled mitophagy by blocking AMPK, and PHF23 knockout could promote the activation of AMPK and enhance mitophagy (Maimaitijuma et al., 2020). Thus, AMPK is an important molecule in the process of autophagy. As an important sensing and regulating device of intracellular energy changes, AMPK plays a key regulatory role in global autophagy and selective mitophagy; it can selectively carry out autophagy and/or mitophagy by sharing some pathways such as AMPK/mTOR and AMPK/ULK1.

It has been found that the intra-articular injection of resveratrol in mice can promote global autophagy through AMPK/mTOR, thereby reducing cartilage degeneration (Qin et al., 2017). In the process of estrogen 17β-estradiol acting on chondrocytes, the AMPK/mTOR signaling pathway is also activated to promote mitophagy and the proliferation of chondrocytes (Mei et al., 2021). This may be due to the enhanced mitophagy that helps eliminate the dysfunctional mitochondria in chondrocytes, which in turn reduces oxidative stress, thereby improving the viability and proliferation of chondrocytes. The sharing of AMPK/mTOR pathways in regulating autophagy and mitophagy allows the fastest removal of damaged organelles and the maximization of aggregates to ensure cell survival. AMPK can target ULK1 downstream molecules to participate in the PINK/PRKN pathway after indirect or direct activation of ULK1 via mTOR, thus playing a role in mitophagy (Egan et al., 2011; Tian et al., 2015; Herzig and Shaw, 2018). AMPK may also contribute to the promotion of mitophagy by directly targeting the PINK/PRKN pathway. It has been found that curcumin can exert a chondroprotective effect in OA rats by promoting mitophagy. In addition, curcumin can maintain mitochondrial homeostasis and normal function (ROS, Ca^{2+}, ATP production, and MMP) by promoting mitophagy (Jin et al., 2022). Further studies have revealed that curcumin may initiate recognition of damaged mitochondria and mitophagy by activating AMPK and subsequently phosphorylating PINK1 in a direct manner (Cao et al., 2020).

Currently, there are few studies related to AMPK-dependent mitophagy. The regulation of autophagy by AMPK is likely to be a broad process, in which there are two types of mitophagy and cell holistic autophagy, and the choice of the specific mode may be determined by the degree of cell stress. This is evidenced by the involvement of AMPK/mTOR and AMPK/ULK1 pathways in regulating autophagy and mitophagy. The key point of mitophagy regulation via AMPK is the activation of the PINK/PRKN pathway by AMPK. In summary, AMPK is an important molecule regulating cellular energy and metabolism, which is closely related to chondrocyte autophagy and mitophagy in OA.

3.1.2 SIRT3 signaling

SIRT3, a member of the Sirtuin protein family, is a nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase located in mitochondria (Lombard et al., 2007). SIRT3 can bind to several important metabolic and respiratory enzymes involved in the regulation of mitochondrial function by deacetylation to maintain mitochondrial function and metabolism (Dai et al., 2014). SIRT3 plays a regulatory role in mitochondrial homeostasis in chondrocytes; the loss of SIRT3 in chondrocytes can lead to mitochondrial dysfunction and the development of OA (He et al., 2020). SIRT3 can also be involved in mediating mitophagy in chondrocytes. The activation of SIRT3 restores the mitochondrial respiratory chain within chondrocytes, thereby maintaining cartilage metabolic balance and protecting mice from post-traumatic OA (Zhang et al., 2023). Furthermore,

SIRT3 deficiency can lead to mitochondrial dysfunction, which can induce OA (Wang JL et al., 2018).

Mitochonic acid-5 (MA-5), a plant hormone indole-3-acetic acid derivative, modulates mitochondrial ATP (Lei et al., 2018). MA-5 can promote the oligomerization of mitochondrial ATP synthase, reduce mitochondrial fragmentation, and restore crista shape and dynamics (Matsuhashi et al., 2017). In recent years, it was found that MA-5 could activate Parkin-dependent mitophagy by upregulating SIRT3 expression, thereby maintaining mitochondrial homeostasis (maintaining MMP and inhibiting mitochondrial division), and block excessive intracellular ROS production in inflammation-induced chondrocytes (Xin et al., 2022). Therefore, MA-5 could maintain normal ROS levels by MQC-mitophagy and inhibit chondrocyte apoptosis. Unfortunately, in this experiment, Parkin expression was detected, while PINK1 expression was not. Based on the hypothesis proposed by Lazarou et al. (2015), PINK1 is the key to the PINK/PARK pathway, and Parkin is more likely to amplify PINK1-initiated mitophagy. Other research has also shown that SIRT3 promotes mitophagy via the PINK/PARK pathway (Wang CZ et al., 2018). Metformin was found to activate SIRT3 expression in inflammatory chondrocytes and promote PINK/PARK-mediated mitophagy, while the SIRT3 inhibitor 3-(1H-1,2,3-triazol-4-yl) pyridine (3-TYP) partially reversed these changes (Wang CZ et al., 2018). Meanwhile, metformin treatment could promote mitochondria fusion level and suppress fission level in chondrocytes, therefore maintaining MMP. In conclusion, metformin-mediated mitophagy maintains mitochondrial homeostasis, thereby inhibiting excess ROS production and ECM degradation in chondrocytes. In addition, irisin is a myokine that is secreted into serum by skeletal muscle after physical exercise. Previous research has found that irisin can stimulate the proliferation of OA chondrocytes and inhibit catabolism (Vadalà et al., 2020). Further research has shown that irisin alleviates oxidative stress and chondrocyte dysfunction stimulated by inflammatory factors by regulating mitochondrial integrity and mitophagy, which may be related to SIRT3/PINK/PARK signaling (Wang FS et al., 2020). In summary, there is currently a lack of sufficient literature to support the direct correspondence between SIRT3 and PINK/PARK, so more experiments are needed to prove this relationship.

Relatively few studies have been conducted on the regulation of SIRT3 on chondrocyte mitophagy, and there is a lack of direct evidence on how SIRT3 regulates PINK/PARK. However, previous research in the field of diabetic cardiomyopathy has found that SIRT3 may regulate mitophagy by increasing the expression of Parkin through the deacetylation of its downstream factor Forkhead box O3a (FOXO3a) (Yu et al., 2017). Some researchers silenced FOXO3a in chondrocytes and found that the low expression of FOXO3a reduced the level of autophagy. Thus, FOXO3a plays an anti-oxidative stress role in chondrocytes (Akasaki et al., 2014).

In summary, combined with previous studies in other disease areas, the above results suggest that SIRT3 regulates mitophagy in chondrocytes either through SIRT3/PINK/PARK or through SIRT3/FOXO3a/PARK. In any case, the role of SIRT3 in regulating chondrocyte mitophagy is beyond doubt.

3.2 PRKN-independent pathway

The PINK1/PRKN pathway has received extensive attention in mitophagy studies. Meanwhile, there are alternatives to this pathway, such as the PRKN-independent pathway. This pathway refers to the ability of certain proteins and lipids to recruit autophagosomes independently of PINK1 and PRKN. It relies primarily on mitochondrial membrane proteins, including B-cell lymphoma-2 (BCL-2)/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3/NIP3), BNIP3-like (BNIP3L)/NIP3-like protein X (NIX), FUN14 domain-containing protein 1 (FUNDC1), FK506-binding protein (FKBP) prolyl isomerase 8 (FKBP8), etc. These membrane proteins are called “mitophagy receptors,” which preferentially interact with different LC3 and GABARAP proteins and subsequently specifically target damaged mitochondria to initiate mitophagy (Novak et al., 2010; Hanna et al., 2012; Liu et al., 2012; Sun et al., 2021).

BNIP3 and BNIP3L are both mitochondrial proteins that belong to BCL2 homology domain 3 (BH3) proteins. As hypoxia-responsive proteins, they are activated by hypoxia-inducible factor 1α (HIF1α) under hypoxic conditions to promote mitophagy (Sowter et al., 2001; Bellot et al., 2009). FUNDC1 is another classical mitochondrial protein, which could interact with ULK1 or phosphoglycerate mutase 5 (PGAM5) to phosphorylate FUNDC1 at Ser17 or Ser13, respectively,

under hypoxia, thereby promoting mitophagy (Liu et al., 2012; Chen G et al., 2014; Wu WX et al., 2014; Chen M et al., 2016). As an OMM receptor, FKBP8 can interact with LC3A, LC3B, GABARAP, and GABARAPL1 (Birgisdottir et al., 2019). FKBP8 can increase the recruitment of LC3A during mitochondrial stress, and thus better promote the occurrence of mitophagy (Bhujabal et al., 2017).

At present, studies on the PRKN-independent pathway of mitophagy in OA mainly focus on BNIP3. It is activated by HIF1α under hypoxic conditions to promote mitophagy (Sowter et al., 2001). Chondrocyte survival under very low oxygen tension is largely dependent on HIF1α (Pfander and Gelse, 2007). Hypoxia stimulates chondrocytes to cause the excessive production of mitochondrial ROS and decrease the MMP. Subsequently, HIF1α/BNIP3-mediated mitophagy promotes chondrocyte survival (Hu et al., 2020). Similar results were found in fibroblast-like synoviocytes (FLSs) from patients with OA and rheumatoid arthritis (RA). Higher expression of HIF1α and BNIP3 in RA-FLS could maintain the redox equilibrium balance of cells through mitophagy and promote cell survival under hypoxic conditions. However, the low level of BNIP3 expression in OA-FLS resulted in reduced levels of mitophagy under hypoxic conditions, which led to a disruption of the mitochondrial redox homeostasis and yielded massive apoptosis (Deng et al., 2022). In addition, some experiments have found that silencing HIF1α can activate intracellular PINK1/PRKN and BNIP3, thereby over-activating mitochondria and caspase/cleaved caspase-3 apoptosis cascade and resulting in apoptosis (Lu et al., 2021). All these experiments demonstrated the importance of HIF1α in the regulation of BNIP3-mediated mitophagy. The presence of HIF1α is essential for chondrocytes and even FLS to survive under hypoxic conditions.

BNIP3 can also respond to AMPK/ULK1 signaling. Trehalose is a novel autophagy activator that can activate the AMPK/ULK1 pathway (Zhang et al., 2014). Some studies have found that trehalose treatment after tert-butyl hydrogen peroxide (TBHP) treatment of chondrocytes can induce BNIP3-mediated mitophagy and endoplasmic reticulum autophagy to play an anti-apoptosis role in chondrocytes (Tang et al., 2017). The active overexpression of BNIP3 can also reduce lipopolysaccharide (LPS)-mediated chondrocyte inflammation and apoptosis by promoting mitophagy (Ma et al., 2020). This anti-apoptotic effect may be played by restoring MMP, inhibiting mitochondrial division, and inhibiting ROS-induced caspase cascade and DNA damage through BNIP3-mediated mitophagy. However, BNIP3 has also been found to promote apoptosis. Previous research established that the knockdown of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) results in the overexpression of BNIP3 and induced mitophagy, leading to cartilage matrix degradation (Kim et al., 2021). It has also been reported that the overexpression of BNIP3 leads to the activation of the Notch signaling pathway, aggravating the condition of OA (Wang WF et al., 2020). This may seem contrary to the previous conclusion, but it is also a reasonable notion; the extent of mitophagy determines whether it promotes apoptosis or survival. Research suggests that only normal mitophagy can play a role in promoting cell survival: both weak and excessive mitophagy can lead to cell apoptosis in the stimulated state.

At present, the research on the relationship between PRKN-independent mitophagy and OA mainly focuses on the BNIP3 protein. This protein can be regulated by HIF1α and the AMPK/ULK1 signaling pathway. Due to the lack of blood supply to the articular cartilage, the deeper layers are more hypoxic. HIF1α/BNIP3-regulated mitophagy was reported to be essential for chondrocyte survival at the deepest part of the growth plate, even when exposed to oxygen tension as low as 1% (Kiaer et al., 1988; Najafipour and Ferrell, 1995). However, the effects of mitophagy on chondrocyte viability need to be specifically analyzed according to the level of mitophagy; high levels of mitophagy can also lead to apoptosis and aggravate OA. In any case, PPKN-independent mitophagy plays a key role in the survival of some cells and tissues without PRKN.

4 Conclusions

OA is a degenerative disease that is closely related to age. During pathogenesis, chondrocytes inevitably develop reduced MMP and mitochondrial dysfunction (Miwa et al., 2022). However, when MMP is reduced to a depolarized state, cells specifically remove depolarized mitochondria through mitophagy, thereby maintaining the homeostasis of mitochondrial function

and cell survival, suggesting that the modulation of mitophagy may be a novel therapeutic strategy for OA. Based on the available evidence, in this paper, the mechanisms and pathway profiles of mitophagy in OA were summarized, including PRKN-dependent and -independent pathways. The activation of PRKN-dependent pathway, which has received widespread attention, is largely dependent on the aberrant accumulation of PINK1 in the OMM and is amplified by signaling from the E3 ubiquitinase Parkin. In this process, multiple upstream molecules, including AMPK and SIRT3, are involved in mediating mitophagy and OA pathogenesis via the PINK/PRKN pathway. The PRKN-independent pathway relies primarily on the interaction of mitochondrial membrane proteins, typified by BNIP3, with ATG8 family proteins to specifically target damaged mitochondria and initiate mitophagy. The deeper chondrocytes are situated, the lower oxygen partial pressure is around them, and hence HIF1α/BNIP3-mediated mitophagy is essential to maintain chondrocyte survival in hypoxic conditions. Notably, whether other PRKN-independent pathways, such as FUNDC1- and BNIP3L-mediated mitophagy, are involved in the regulation of OA lesions is unclear, suggesting new research directions regarding mitophagy and OA.

Mitophagy is an important mechanism regulating chondrocyte survival and thus affecting the progression of OA. As such, targeted therapies that modulate mitophagy may offer new hope for the clinical treatment of OA. Several potential drugs have been identified that can exert chondroprotective effects by increasing mitophagy, such as algin (Tang et al., 2017), curcumin (Jin et al., 2022), metformin (Wang CZ et al., 2018), irisin (Vadalà et al., 2020), estrogen 17β-estradiol (Mei et al., 2021), and MA-5 (Lei et al., 2018). However, these drugs may exert chondroprotective effects through other pathways while activating mitophagy. In addition, the mode of administration and the effective time window of these drugs need to be further investigated. Therefore, the continued exploration of autophagy-regulated drugs is needed to further clarify the specific mechanisms by which these drugs exert a cartilage protection effect and their applications to ensure their feasibility and efficacy. Intensive studies of mitophagy may shed light on the treatment of OA as well as other aging-related degenerative diseases.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 82071762), the Shanghai Key Lab of Human Performance (Shanghai University of Sport) (No. 11DZ2261100), and the 2021 Capacity Building of Shanghai Universities (No. 21010503600), China.

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Compliance with ethics guidelines

Hong CAO, Xuchang ZHOU, Bowen XU, Han HU, Jianming GUO, Miao WANG, Nan LI, and Jun ZOU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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