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Autophagy and its significance in periodontal disease

Yuhui Yang | Yiping Huang | Weiran Li

Department of Orthodontics, Peking University School and Hospital of Stomatology, Beijing, China

Correspondence
Yiping Huang and Weiran Li Department of Orthodontics, Peking University School and Hospital of Stomatology, Beijing 100081, China.
Emails: hyp891213@163.com and weiranli@bjmu.edu.cn

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Abstract

Autophagy is an evolutionarily conserved process essential for cellular homeostasis and human health. As a lysosome-dependent degradation pathway, autophagy acts as a modulator of the pathogenesis of diverse diseases. The relationship between autophagy and oral diseases has been explored in recent years, and there is increasing interest in the role of autophagy in periodontal disease. Periodontal disease is a prevalent chronic inflammatory disorder characterized by the destruction of periodontal tissues. It is initiated through pathogenic bacterial infection and interacts with the host immune defense, leading to inflammation and alveolar bone resorption. In this review, we outline the machinery of autophagy and present an overview of work on the significance of autophagy in regulating pathogen invasion, the immune response, inflammation, and alveolar bone homeostasis of periodontal disease. Existing data provide support for the importance of autophagy as a multi-dimensional regulator in the pathogenesis of periodontal disease and demonstrate the importance of future research on the potential roles of autophagy in periodontal disease.

KEYWORDS

alveolar bone resorption, autophagy, inflammation, periodontal disease, periodontal pathogens, the immune response

1 | INTRODUCTION

Autophagy is a highly conserved catabolic process in which cellular components such as misfolded proteins or damaged organelles are sequestered into lysosomes for degradation.[1,2] The target materials of autophagy are recycled to create new cellular structures, or alternatively used as a source of energy.[3] Autophagy can be stimulated by multifarious environmental stresses including hypoxia, nutrient deprivation, oxidative stress, or intracellular pathogens. It is essential for cellular homeostasis, which accurately responds to stimuli in the absence of energy or nutrients to prevent cell damage.[4] It also participates in various biological processes, such as cellular differentiation, cell function, and defense against pathogens.[5] In addition, autophagic dysfunction is associated with multiple diseases such as autoimmune disease, cancer, diabetes, and oral disease.[2,6]

Periodontal disease (PD) is a chronic inflammatory disease affecting tooth-supporting tissues, including gingiva, periodontal ligament, and alveolar bone. It is common in populations worldwide and highly prevalent in adults.[7] Microorganisms of the dental plaque are considered to be the initial pathogenic factor of PD. Disease occurs when the balance between pathogens and the host immune response is disrupted.[8] The host overcorrection to microbial infection results in a local inflammatory state, leading to the progressive destruction of periodontal ligament and alveolar bone.[9,10] In this review, we examine autophagy and its significance in periodontal disease. We begin by briefly reviewing the autophagy machinery, followed by a review of updated information on the potential role of

Yiping Huang and Weiran Li contributed equally to the work.

autophagy in periodontal pathogens, the immune response, inflammation, and alveolar bone homeostasis.

2 | AUTOPHAGY TYPES AND MACHINERY

Three primary types of autophagy have been described: microautophagy, chaperone-mediated autophagy, and macroautophagy. The types of autophagy mainly differ in the mode of cargo delivery to the lysosome. In microautophagy, the cargo interacts directly with the lysosome surface and is subsequently cleaved by proteases.[11] Microautophagy is thought to be involved in long-lived protein turnover in mammalian cells.[12] Chaperone-mediated autophagy is a highly selective type of autophagy that relies on translocation of pertinent soluble cytosolic proteins across the lysosomal membrane.[13] In the strictest and best characterized form of autophagy, "macroautophagy" (referred to hereafter as autophagy), recycling of cellular materials is dependent on specialized cytosolic vesicles under the control of autophagy y-related (ATG) genes.[14] The autophagic process can be divided into various steps: initiation, elongation, enclosure, fusion with lysosomes, and degradation (Figure 1). The initiation step begins with the formation of phagophores; both ends of the membrane of phagophores elongate to engulf and enclose the targeted materials within double-membrane autophagosomes.[14,15] The maturation of autophagosomes occurs when they are fused with lysosomes to become autolysosomes. The inner membrane of autolysosomes and engulfed materials are degraded by lysosomal acid proteases.[4,14]

The molecular machinery of autophagy was discovered in yeast genetic studies in which more than 30 ATG genes have been identified.[16] Many orthologues of ATG genes have been identified in mammalian cells. The corresponding gene products are required for the dynamic processes of autophagy. Under stress conditions, the mammalian target of rapamycin (mTOR) is inactivated, which consequently activates ATG1 kinase activity.[17] UNC-51-like kinase (ULK)/ATG1 is a key protein that initiates autophagy by forming a complex with ATG13, ATG101, and focal adhesion kinase family interacting protein of 200 kD (FIP200) to induce autophagic signals.[4,18] The class-III phosphatidylinositol 3-kinase (PI3K) complex composed of Beclin1/ATG6, vesicular protein sorting (Vps) 34, Vps15, and ATG14, induces the production of phosphatidylinositol-3-phosphate to recruit effectors required for the formation of autophagosomes.[19,20] ATG9 is a transmembrane protein and its bidirectional movement between phagophore assembly sites (PASs) and non-PASs contributes to the delivery of membranous structures to form autophagosomes.[21] Two ubiquitin-like conjugation systems are also required for the vesicle elongation process. One system is mainly formed by ATG5 and ATG12; the other involves the conjugation of phosphatidylethanolamine (PE) to microtubule-associated protein 1 light chain 3 (LC3)/ATG8.[22] LC3 is synthesized as pro-LC3, which is cleaved at the C-terminus with the help of protease AGT4 to form LC3-I. The lipid conjugation of PE leads to the conversion of LC3-I to the autophagic membrane-bound LC3-II.[23] LC3-II is recruited to the membranes of autophagosomes and remains on the completed autophagosomes until lysosomal fusion.[3,24]

3 | AUTOPHAGY AND PERIODONTAL PATHOGENS

Numerous microorganisms and their metabolic products compose the dental plaque.[25,26] Over 500 microbial species have been

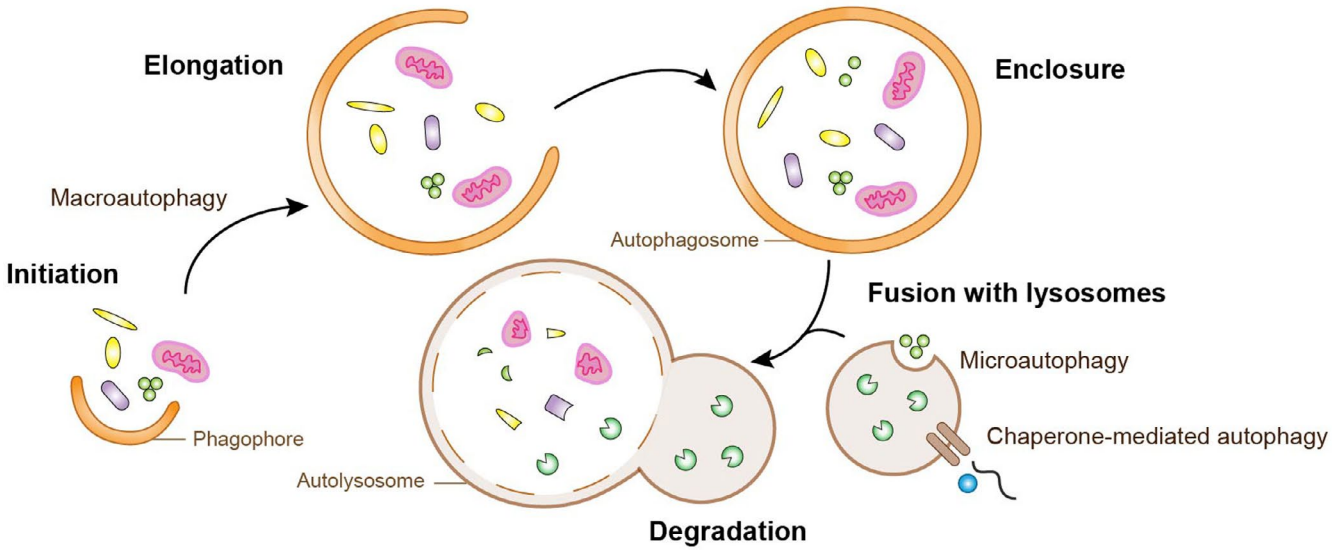

FIGURE 1 Schematics showing autophagic flux. Upon initiation of macroautophagy, cytosolic materials are encapsulated by a double membrane. Both ends of the phagophore elongate, resulting in the formation of an autophagosome. The outer membrane of the autophagosome then fuses with a lysosome to form an autolysosome, where the contents are degraded. Microautophagy occurs when the cytosolic cargo is directly sequestered by invagination of the lysosomal membrane. In chaperone-mediated autophagy, substrate proteins are recognized by lysosomal chaperones and transported across the lysosomal membrane through the membrane receptor

identified from the human dental plaque thus far, and the dysbiosis of microbial biofilms is considered a pathogenic precursor of PD.[27] In periodontitis, subgingival plaque is mainly composed of Gram-negative periodontal bacteria, including *Porphyromonas gingivalis* (P.g), *Aggregatibacter actinomycetemcomitans* (A.a), *Tannerella forsythia*, *Treponema denticola*, and Spirochetes. Among these, P.g is a major opportunistic pathogen associated with PD that has been widely studied.[28,29]

Autophagy has a dual role in response to periodontal pathogens. First, autophagy enhances the survival of periodontal pathogenic bacteria. Increasing evidence indicates the ability of host-adapted pathogens to exploit host autophagy for survival and persistence in the host.[30,31] Some periodontal bacteria have evolved mechanisms that use the autophagic response; therefore, autophagy may provide a route for bacteria to escape from the host immune defense. It has been confirmed that the level of autophagy is higher in patients with periodontitis than in healthy individuals.[32,33] As an inducer of PD, P.g and its lipopolysaccharide (LPS) have been demonstrated to enhance autophagic activity.[32,34-36] A study found that the inhibition of autophagy by 3-methyladenine (3-MA) or ATG5 depletion significantly decreased the survival of P.g in gingival epithelial cells (GECs).[34] PI3K/ protein kinase B (Akt)/mTOR signaling pathway is a critical regulator of autophagy and inactivation of it results in autophagy after P.g invading.[35] The induced autophagy in GECs provides a favorable microenvironment for its persistence and evasion of immune defense.[37,38] Moreover, P.g in the cytosol is usually degraded by lysosomes, but the ratio of free P.g in the cytosol is low compared with P.g co-localized with double-membrane vacuoles.[39] Thereby, most co-localized P.g evades host defenses by impairing the formation of autolysosomes and subsequently accumulated autophagosomes supply nutrients for its survival.[34,37] Interestingly, a recent study found that P.g manipulated the autophagic process to escape from immune surveillance and survived within dendritic cells (DCs).[40] The activation of Akt/mTOR signaling axis suppressed antimicrobial autophagic machinery, resulting in survival of intracellular P.g.[40]

By contrast, autophagy also induces a type of cell death against infection by periodontal bacteria. Butyrate, a metabolic by-product of periodontal bacteria, promoted cell death via autophagy induction by increasing the conversion of LC3-I to LC3-II in GECs. The autophagy inhibitor 3-MA significantly suppressed cell death induced by butyrate.[41] Furthermore, the induction of autophagy in immune cells enhanced intracellular bacteria killing during the antibacterial process.[42,43] The relationship between autophagy and periodontal pathogens is complex during the pathogenesis of PD and requires further investigation.

4 | AUTOPHAGY AND THE IMMUNE RESPONSE

The progression of PD is influenced by multiple factors and the imbalance between host immunity and pathogens is one of the crucial factors for PD.[44,45] Activation of the innate immune response is considered as the first line of defense against bacterial infection. Pattern recognition receptors (PRRs) including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain -like receptors (NLRs) on the surface of phagocytes are critical components necessary to trigger the primary immune response. PRR signaling is induced following the recognition of pathogen-associated molecular patterns and damage associated molecular patterns after pathogen attack of host tissues.[46]

Autophagy restricts pathogen invasion and serves as an effector mechanism of innate immunity.[47] It provides a mechanism for the elimination of invading pathogens in immune cells. Phagocytes, including macrophages, neutrophils, and monocytes, are key cells of the innate immune response that initiate a timely response against a large number of pathogens. The enhanced autophagic flux induced by periodontal bacteria is found in periodontal tissues as well as in these immune cells. After invasion of P.g, the expression of autophagy-related Beclin1 and LC3-II, and ATG5-ATG12 conjugation was increased in THP-1-derived macrophages and inhibition of autophagy resulted in deceased P.g killing.[42] The autophagic response was induced following A.a infection in THP-1 cells, which inhibited the intracellular survival of A.a.[43] Moreover, the induction of autophagy by rapamycin impaired P.g survival within DCs.[48]

The interaction between TLRs and autophagy amplifies the effects of both systems in response to pathogen invasion in the periodontium. Two members of the TLR family, TLR2 and TLR4, are essential for the pathogenesis of PD because the ligands of these receptors are components of periodontal bacteria.[49] Increased expression of TLR2 and TLR4 was observed in gingival epithelia and underlying connective tissues in chronic periodontitis.[50-52] Furthermore, alveolar bone resorption induced by P.g infection was not observed in TLR4 knock-out mice.[53,54] TLRs induce autophagy early to ensure the timely upregulation of antimicrobial activities. The ubiquitylation of ULK1 and Beclin1 by tumor necrosis factor receptor–associated factor (TRAF) 6 results in amplification of TLR4-induced autophagy.[55] Studies found that an increased level of autophagy was induced with TLR ligands in macrophages and the induction of autophagy inhibited the viability of intracellular pathogens.[56,57] TLR signaling increased autophagic flux and TLR1/2 agonists inhibited the survival of P.g within human DCs.[48] Recently, Wei et al found that activation of TLR9 initiated the autophagic response in macrophage cell lines and the trend of TLR9 expression changes was the same in periodontitis and autophagy, suggesting that TLR9 was closely associated with autophagy in periodontitis.[58]

In addition to the innate immune response, adaptive immune cells and cytokines are also important players in the pathogenesis of PD. The cross-talk between the innate and adaptive immune response plays an important role in PD.[59] Autophagy functions as a regulator of antigen presentation and T-cell function in the adaptive immune response. The T-cell-mediated immune response is dependent on antigen-presenting cells including DCs, which are crucial for initiation of the adaptive immune response. Autophagy has been shown to be active in DCs.[60,61] Autophagy proteins in DCs are critical for the fusion of lysosomes with antigens during

presentation, suggesting autophagy is involved in enhancing antigen presentation.[60,62] Furthermore, autophagy influences the adaptive immune response in the differentiation, metabolism, and function of T cells.[46,63] There are at least four types of CD4+ T cells (T-helper cells), including T-helper 1, T-helper 2, T-helper 17, and T-regulatory cells; these cells participate in the adaptive immune response to various pathogens.[8] In an ATG3-deficient mouse model, CD4+ T cells were decreased in the spleen and lymph nodes.[64] Upon Beclin1 deletion, decreased numbers of CD4+T cells were observed due to the increase in cell death proteins.[65] Based on these data, we conclude that autophagy is essential for regulating the innate and adaptive immune responses in the defense against pathogens.

5 | AUTOPHAGY AND PERIODONTAL INFLAMMATION

The initial inflammation in periodontal tissues following pathogen invasion is a physiologic defense mechanism.[66] The goal of an effective immune response is to resolve the acute inflammation and establish periodontal tissue homeostasis.[67] If a defective or overactive immune response results in a prolonged inflammatory response, the inflammation extends deep into the connective tissues. The aberrant induction of inflammation breaks the balance between pro-inflammatory and anti-inflammatory mechanisms in the periodontium.

Under inflammatory conditions, activation of autophagy protects cells from apoptosis. An inflammatory environment composed of tumor necrosis factor (TNF)-α and interleukin (IL)-1β enhanced the expression of autophagy protein LC3-II, Beclin1, and ATG12 in periodontal ligament stem cells (PDLSCs).[68] The level of autophagy was significantly increased in PDLSCs treated with TNF-α, while apoptosis was suppressed.[33] Moreover, inhibition of autophagy using 3-MA increased apoptosis in human gingival fibroblasts (HGFs).[32] Autophagy and apoptosis often occur in the same cell and the induction of autophagy contributes to the suppression of apoptosis.[69] Thus, autophagy may provide a protective effect in an inflammatory environment. P38 mitogen-activated protein kinase (MAPK) pathway is required for autophagy and is shown to induce autophagy in HGFs after endoplasmic reticulum stress resulting from PD.[70]

The role of the autophagic response in the promotion of angiogenesis has been investigated in the periodontium. Abnormal angiogenesis is a significant feature of periodontal inflammation.[71] Mesenchymal stem cells (MSCs), including PDLSCs, have been shown to promote angiogenesis, in which autophagy plays a role.[72-74] The secretion of angiogenin (Ang) and basic fibroblast growth factor (bFGF), two important angiogenesis-promoting cytokines, was upregulated under inflammatory conditions.[68] Induction of autophagy with rapamycin and Beclin1 overexpression upregulated the level of Ang and bFGF in PDLSCs, whereas knockdown of Beclin1 suppressed angiogenesis-promoting ability.[68]

Autophagy has been found to inhibit the secretion of pro-inflammatory factors and the formation of inflammasomes. The anti-inflammatory function of autophagy was discovered from the observation that the production of IL-1β and IL-18 was increased in the absence of functional ATG16L1 in Crohn's disease.[75] IL-1, especially IL-1β, amplified the host inflammatory response in PD.[76] Autophagic activity is responsible for sequestering pro-IL-1β from caspase 1 in autophagosomes. IL-1β recruits downstream TRAF6 and activates TRAF6-dependent ubiquitylation of Beclin1.[77] The induction of autophagy with rapamycin leads to the loss of pro-IL-1β and inhibition of IL-1β secretion in LPS-treated antigen-presenting cells.[78] An inflammasome is a multiple protein complex involved in the recognition of microorganisms, which stimulates the release of mature IL-1β. Several studies have shown that autophagy plays a negative role in inflammasome activation.[79,80] The ubiquitination of assembled inflammasomes and the recruitment of p62 serve to link inflammasomes to autophagy.[80] Furthermore, inhibition of autophagy significantly increased IL-1β release and NLR family pyrin domain containing 3 (NLRP3) inflammasome formation, suggesting that autophagy limited the P.g-induced inflammatory response.[42] High levels of mitochondrial ROS production which damage the cell integrity and function have been shown in patients with periodontitis and a reduction in autophagosome formation was observed after ROS enhancement in HGFs.[32,81] Consistent with previous findings, ROS induction by the autophagic inhibitor 3-MA contributed to NLRP3 inflammasome activation and increased IL-1β production.[82,83] Another study showed that induction of autophagy decreased ROS accumulation in LPS-stimulated HGFs.[84] Furthermore, a study showed that A.a-induced autophagy limited excessive inflammation via inhibition of the release of IL-1β and ROS in macrophages.[83] A.a activated the influx of autophagy by increasing the expression of the TLR and extracellular signal-regulated kinase(ERK) signaling pathways.[83]

Several autophagy-related molecules are also involved in the regulation of periodontal inflammation. Cathepsin S (CTSS) is a lysosomal multifunctional cysteine protease and a critical regulator of autophagy.[85,86] CTSS is a hub protein in the protein-protein interaction network involved in the development of periodontitis.[87] Memmert et al showed that IL-1β-induced inflammatory conditions significantly upregulated CTSS expression in human fibroblast-like periodontal ligament cells. They also found that CTSS inhibited autophagy and inhibition of CTSS-induced autophagy through ROS-mediated PI3K and c-Jun N-terminal kinase (JNK) signaling pathways.[88] Damage-regulated autophagy modulator (DRAM) 1 is a lysosomal membrane protein that promotes autophagy to function against intracellular pathogens in a p53-dependent manner.[89,90] DRAM1 gene expression increased significantly in response to IL-1β in vitro and both mRNA and protein levels of DRAM1 were higher in gingival biopsies of periodontitis patients.[91]

6 | AUTOPHAGY AND ALVEOLAR BONE HOMEOSTASIS

Alveolar bone homeostasis is maintained by the balance between osteoclastogenesis and osteoblastogenesis.[92] An imbalance favoring bone resorption over formation results in alveolar bone resorption

in periodontitis. Due to the large amounts of waste materials including damaged organelles, and mineral and organic components of the bone matrix during bone resorption, dynamic autophagy is required for degradation and recycling of damaged intracellular structures. Autophagy-related proteins have been demonstrated to be important mediators in the differentiation and function of bone cells in physiologic and pathologic conditions, suggesting a crucial role of autophagy in bone homeostasis.[93]

6.1 | Osteoclasts

Osteoclasts are multinucleated cells derived from hematopoietic stem cells upon stimulation with macrophage colony-stimulating factor and receptor activator of nuclear factor kappa-B ligand. Increasing evidence has revealed that autophagy regulates osteoclast differentiation and bone resorption.[94,95] AMP-activated protein kinase (AMPK)/mTOR/p70 ribosomal protein S6 kinase (p70S6K) signaling pathway is involved in autophagy activation and the regulation of synthesis and decomposition of osteoclast metabolic processes.[95] Osteoclasts resorb bone via the ruffled border. Lysosomal fusion with the plasmalemma results in the release of matrix-degrading molecules such as cathepsin K into the extracellular space to digest bone matrix. Autophagy-related proteins including ATG5, ATG7, ATG4β, and LC3 are essential for generation of the osteoclast ruffled border and secretion of lysosomal enzymes in vivo and in vitro.[96]

Autophagy is responsible for increasing the number of osteoclasts and for persistent osteoclastic activation during alveolar bone resorption in PD. LPS stimulates osteoclast differentiation by enhancing autophagy and increasing ROS levels in pre-osteoclasts.[97] ROS contributes to osteoclast activation during the host response, resulting in pathological bone destruction.[98] Overexpression of Beclin1 significantly increased the number of tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts, whereas the inhibition of autophagy inhibited osteoclastogenesis.[99] Recently, He et al showed that the number of TRAP-positive multinucleated cells was lower in a group using autophagy inhibitors 3-MA or chloroquine. Furthermore, 3-MA downregulated LPS-induced osteoclast formation in a periodontitis rat model.[100]

Studies have shown that autophagy has a positive effect on osteoclast activity in response to pro-inflammatory cytokines. The pro-inflammatory cytokine IL-17A contributes to the pathogenesis of periodontitis, especially alveolar bone loss.[101,102] Song et al found that IL-17A facilitated osteoclast differentiation and exacerbated bone resorption in vitro and in vivo and upregulated autophagy activity, including LC3 levels and autophagosome formation. Furthermore, the autophagy inhibitor 3-MA decreased the levels of osteoclast-related markers.[103] ATG7-deficient osteoclast precursors did not exhibit IL-1β-mediated upregulation of cathepsin K secretion.[104] Thus, inhibition of osteoclast activation is a potential approach for protecting alveolar bone from excessive resorption in PD.

6.2 | Osteoblasts and osteocytes

The main function of osteoblasts is to mineralize and synthesize the bone matrix. Autophagy plays an essential role in the differentiation and mineralization of osteoblasts. As an autophagy receptor targeting ubiquitinated proteins for degradation, neighbor of BRCA1 (NBR1) negatively regulates osteoblast differentiation and function.[105] Whitehouse et al demonstrated that genetic truncation of murine NBR1 increased osteoblast differentiation and activity in vivo leading to activation of p38 MAPK.[106] FIP200, an essential component of the autophagic process, enhanced osteoblast nodule formation and differentiation.[107] Nollet et al found that the autophagy proteins ATG7 and Beclin1 were essential for mineralization in an osteoblastic cell line, and ATG5 deficiency in osteoblasts resulted in decreased bone volume in vivo.[108] Inhibition of autophagy also negatively regulated MSC differentiation into osteoblasts.[109,110] Coordinated AMPK-dependent autophagy and Akt/mTOR activation were crucial for osteoblastic differentiation and maintenance of bone mass.[110] A recent study found that autophagy induced by rough surfaces of dental implants accelerated the transition from osteoblast proliferation to maturation and the subsequent differentiation into osteocytes.[111] Moreover, anti-inflammatory action enhanced autophagy and suppressed apoptosis of osteoblasts, suggesting that autophagy of osteoblasts alleviates bone loss associated with inflammation.[112,113]

Osteoblast differentiation is accompanied by changes in cell morphology and intracellular organelle contents. Terminally, osteoblasts transform into osteocytes and localize to mineralized bone matrix.[114] Upregulation of autophagic flux may be a mechanism for differentiated osteocytes to survive in the hypoxic and poor nutrient conditions within the bone matrix. The increased autophagy provides raw materials for osteocytes to adapt to a stressful environment. Using murine osteocytic cell lines, Zahm et al demonstrated that differentiated osteocyte-like cells exhibited elevated levels of LC3-II and autophagosomes.[115] They also observed punctate distribution of LC3 in osteocytes, which was not observed in osteoblasts on the bone surface in rat tibia.[115] Another study demonstrated that decreased autophagy via deletion of ATG7 led to lower bone mass y.[116]

7 | CONCLUSIONS

Autophagy plays a dual role in the protection and elimination of periodontal pathogens in the pathogenesis of PD. In recent years, researchers in this field uncovered a new layer of complexity in terms of how autophagy functions as a regulator of host inflammatory and immune responses to periodontal pathogenic bacterial stimuli. Increasing data provide evidence for an essential role of autophagy in regulating the differentiation and function of bone cells in alveolar bone resorption. This review highlights the multiple regulatory effects of autophagy on periodontal pathogens, the immune response, inflammation, and alveolar bone homeostasis in the development

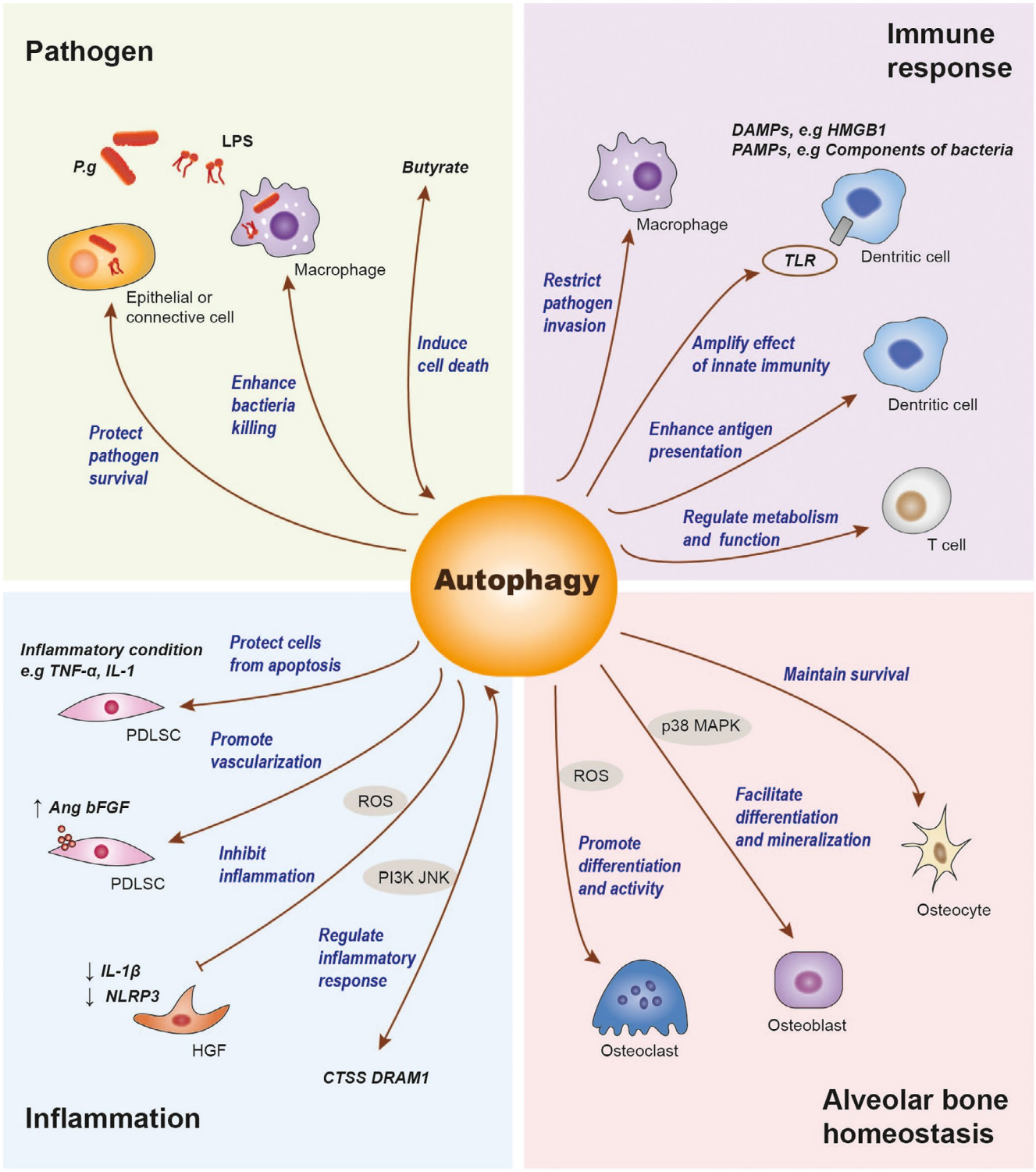

FIGURE 2 Autophagy and its significance in periodontal disease. An overview of the potential roles of autophagy in the pathogenesis of periodontal disease. Autophagy enhances innate and adaptive immune responses, inhibits inflammation, and facilitates alveolar bone mineralization in terms of preventing periodontal disease. On the other hand, autophagy activation might contribute to the development of periodontal disease as it provides a route for microbial survival in the periodontium and allows osteoclastic activation to resorb alveolar bone

of PD (Figure 2). Maintaining autophagic homeostasis could be a potential therapy for controlling host responses and alveolar bone resorption of PD in the future. However, the interaction between autophagy and PD is still not well understood. More work is needed to uncover new ways in which this conservative self-defensive machinery functions in PD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Yuhui Yang https://orcid.org/0000-0002-8197-4979
Yiping Huang https://orcid.org/0000-0003-2263-0612
Weiran Li https://orcid.org/0000-0002-6635-6881

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