Osteoporosis and autophagy: What is the relationship?

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Study conducted at Universidade Federal de São Paulo – Escola Paulista de Medicina (Unifesp-EPM); Faculdade de Odontologia da Universidade Estadual Paulista (Unesp); Faculdade de Medicina da Universidade de São Paulo (FMUSP), SP, Brazil

Article received: 5/10/2016
Accepted for publication: 5/31/2016

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http://dx.doi.org/10.1590/1806-9282.63.02.173

Summary

Autophagy is a survival pathway wherein non-functional proteins and organelles are degraded in lysosomes for recycling and energy production. Therefore, autophagy is fundamental for the maintenance of cell viability, acting as a quality control process that prevents the accumulation of unnecessary structures and oxidative stress. Increasing evidence has shown that autophagy dysfunction is related to several pathologies including neurodegenerative diseases and cancer. Moreover, recent studies have shown that autophagy plays an important role for the maintenance of bone homeostasis. For instance, in vitro and animal and human studies indicate that autophagy dysfunction in bone cells is associated with the onset of bone diseases such as osteoporosis. This review had the purpose of discussing the issue to confirm whether a relationship between autophagy dysfunction and osteoporosis exits.

Keywords: autophagy, bone tissue, osteoblast, osteocyte, osteoclast, osteoporosis.

Introduction

Bone tissue is constantly being remodeled by the coordinated action of osteoclasts, osteoblasts, osteocytes, and bone lining cells.[1,2] Osteoclasts and osteoblasts are respectively responsible for bone resorption and formation, while osteocytes are important in maintaining and controlling bone remodeling.[2-5] Bone remodeling is necessary for the repair of microfractures, as well as for skeletal adaptation to different mechanical stimuli and for calcium homeostasis.[6] However, any imbalance in this process so that bone reabsorption exceeds formation can result in bone loss and subsequent osteoporosis.[7-10]

Osteoporosis is a systemic bone disease characterized by progressive loss of bone mass, bone fragility and susceptibility to fractures.[11] The incidence of osteoporotic fractures, associated with morbidity, mortality and the costs of these fractures, has increased substantially and became a public health problem in many countries,[12-14] including Brazil.[15-17] As life expectancy increases, the number of osteoporotic fractures is expected to increase from 1.7 million in 1990 to 6.3 million in 2050.[18]

The cause of osteoporosis is multifactorial and includes genetic, hormonal and nutritional factors, combined with people's lifestyle choices.[19,20] Peak bone mass is reached early in adult life and from this point both men and women start to lose bone mass more or less depending on a combination of intrinsic and extrinsic factors.[21,22] This process can be aggravated by a lack of physical exercise,[23] prolonged treatment with corticoids,[24] estrogen deficiency, especially in postmenopausal women,[25] presence of other chronic diseases, and by aging.[20]

Recent evidence has pointed out that autophagy, a cell survival pathway, plays an important role in the maintenance of bone homeostasis,[26-28] and changes in this pathway have been related to osteoporosis.[29,30] Since osteoporosis became a serious public health problem with an incidence rate that is likely to increase in the next decades, understanding the cellular and molecular mechanisms involved in the process of bone loss is crucial and paramount for the development of new therapies.

In this review, we discuss studies that associate the autophagic pathway with osteoporosis, aiming to better

understand if there is a possible correlation between these two processes.

Autophagy

Autophagy (from the Greek *autos*, self, and *phagein*, eat) is a lysosomal degradation pathway responsible for the degradation and recycling of cellular components such as unnecessary organelles and proteins, also serving to destroy intracellular pathogens.[31-33] Autophagy occurs in every cell so that structures that are unnecessary to the cell can be recycled. However, factors that induce cellular stress, such as hypoxia, caloric restriction and the accumulation of oxidative stress, can induce autophagy.[34-36]

Three types of autophagy are described: macroautophagy, microautophagy and chaperone-mediated autophagy. The latter involves direct translocation of cytoplasmic proteins into lysosomes, aided and directed by chaperone proteins.[37] In microautophagy, invagination of the lysosomal membrane results in small portions of the cytoplasm being captured towards the lysosome lumen.[38,39] Macroautophagy, hereafter referred to as autophagy, is the most studied of the three autophagic pathways, it has been maintained throughout evolution, from yeast to mammals, and is characterized by the formation of autophagosomes.[40,41] After autophagic stimulation, a small vesicle called the phagophore elongates and subsequently surrounds a portion of the cytoplasm, resulting in the formation of a double membrane structure, termed autophagosome.[31,42] The membrane of the autophagosome then fuses with a lysosome, resulting in the degradation of the entrapped material. Amino acids, fatty acids and other molecules resulting from degradation return to the cytoplasm for recycling and energy production.[38,42]

Usually, the autophagic process is divided into four main steps: initiation/nucleation, elongation, maturation and degradation.[38,42] During initiation, which takes place after autophagic stimulus, a membrane known as phagophore is formed. Phagophore formation and nucleation occur with the formation of two multiprotein complexes: one is composed of proteins ULK1/2, Atg13 and FIP200, and the other, which is known as class III phosphatidylinositol 3-kinase (PI3K) complex, is formed by proteins beclin-1, Vps15, Vps34, Ambra1, UVRAG, and more.[33,43] These complexes, with the participation of several other proteins, are responsible for recruiting and initiating phagophore elongation.[44] Beclin-1 is fundamental for the formation of PI3K complexes and, therefore, has been commonly used as a marker of autophagia.[45]

After nucleation, phagophore elongation occurs and an autophagosome is formed. This process is coordinated by two protein complexes called ubiquitin-like conjugation system, formed by several Atgs (autophagy-related genes) proteins, including Atg3, Atg4, Atg5, Atg7, Atg10, Atg12 and Atg16. This system facilitates conjugation of a molecule called LC3 (a light chain of the microtubule-associated protein 1) with phospholipid phosphatidylethanolamine to LC3II. LC3II then enters the autophagosome's membrane, assisting its elongation and closure.[38,46] Therefore, it is currently known that LC3II is found within the autophagosome's membrane, being key to its formation. Because of that, LC3II has been widely used as a specific autophagosome marker.[38,47]

The other stages of autophagy consist in autophagosome maturation and degradation of its contents. Autophagosome maturation refers to its fusion with components of the endocytic pathway, such as early and late endosomes and lysosomes, turning the contents inside the autophagosome acid.[48] Several proteins involved in vesicular transport, such as dynein, and membrane fusion in the endocytic pathway, including Rab7, SNARES and ESCRT, beclin-1, Vps34 and UVRAG, are involved in autophagosome maturation.[49] The last stage of the autophagic pathway is that of degradation of the autophagosome's contents after fusing with a lysosome and subsequent reuse of the resulting molecules (lipids, amino acids, nucleotides, etc.) as a manner of recycling cell components and producing energy.[50]

For a long time, autophagy was considered a pathway of non-selective degradation.[48] However, it is currently accepted that this process can be extremely specific since organelles are selectively targeted for degradation.[51] The most widely known example of protein-specific autophagic degradation is that of protein p62 (ou SQSTM1), which binds to ubiquitinated proteins leading them to the interior of autophagosomes.[52] This mechanism is mediated by binding of p62 to LC3II present in the autophagosome's membrane, so that p62 is also internalized and ultimately degraded. This protein is thus considered as one of the main substrates for autophagosomes and, therefore, its increased expression indicates decline in the autophagic process and vice versa. Analyses of the combined expression of proteins p62 and LC3II are commonly used to assess the autophagic flow in cells.[53,54] A summary of the major stages of the autophagic pathway is illustrated in Figure 1.

Role of autophagy in bone biology

In recent years, a growing number of studies has shown that autophagy plays an important role to maintain bone homeostasis.[26,27,55] For example, cultures of autophagy

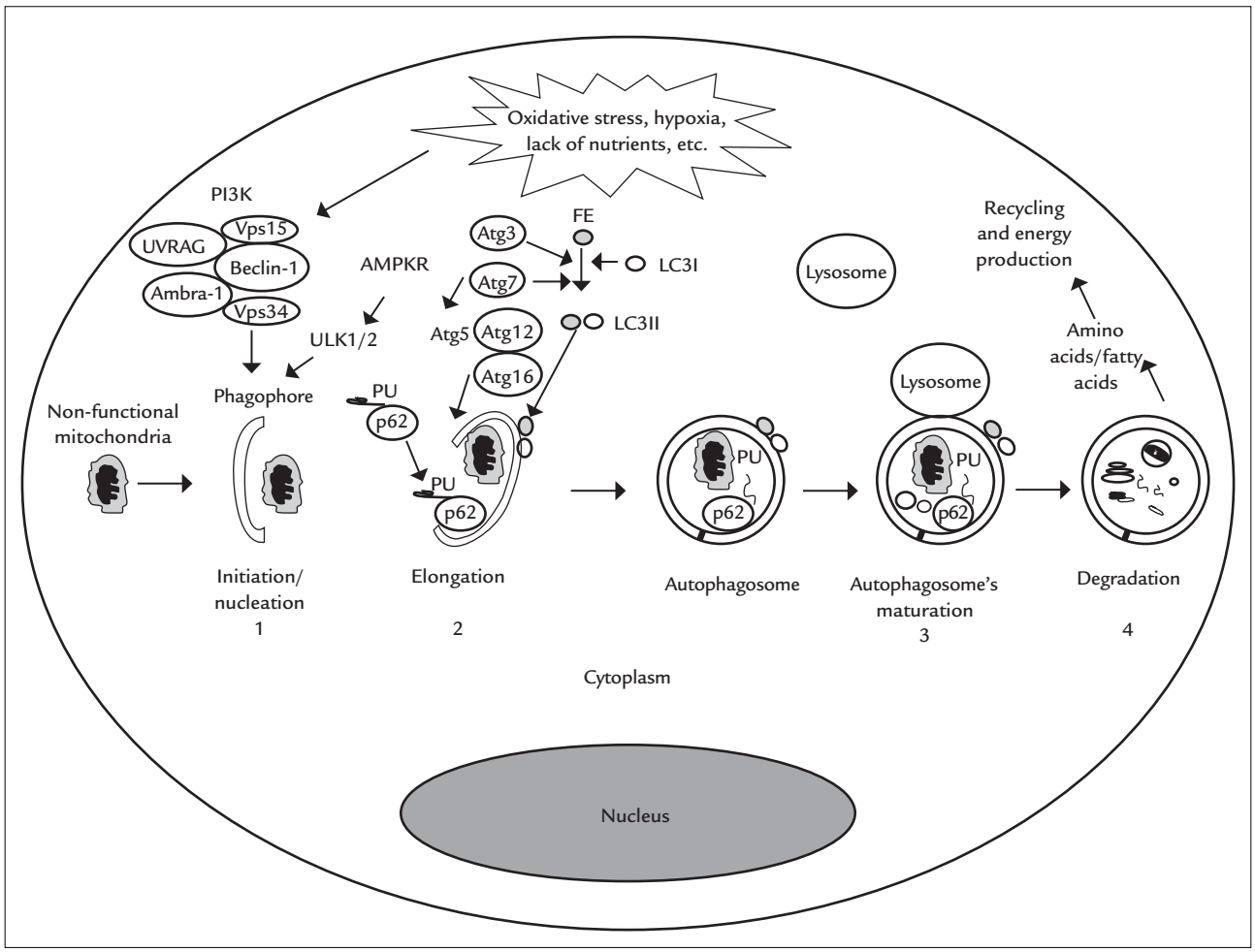

FIGURE 1 Schematic model of the four main stages of the autophagic pathway: 1. initiation/nucleation; 2. elongation; 3. maturation; and 4. degradation. After autophagic stimulation (oxidative stress, hypoxia, lack of nutrients, etc.), phagosome formation/nucleation is observed (1), assisted by multiprotein complexes PI3K (formed by beclin-1, Vps34/15, Ambra-1 and UVRAG) and ULK1/ULK2, as well as Atgs. Assisted by protein LC3II, the phagophore elongates and circles organelles and proteins that are no longer functional (2), including polyubiquitinated proteins (PU) carried by p62, forming the autophagosome. The latter may fuse with a lysosome (3), so that its contents are degraded (4).

gene-knockout osteoblasts (*BECN-1, ATG7* e *LC3*) result in deficiency in the process of mineralization.[56]

In vitro studies show that pharmacological inhibition of autophagy in osteoblast-like cells increases oxidative stress and stimulates apoptosis in these cells. In contrast, induction of autophagy in cultures of osteoblast-like cells reduces their oxidative stress and inhibits apoptosis.[57] It has also been reported that estrogen inhibits osteoblast apoptosis in vitro, inducing autophagy in these cells.[58]

Autophagy is also essential when osteoblasts are incorporated into the bone matrix, thus becoming osteocytes.[59] During the transition, osteoblasts undergo evident changes in their shape, accompanied by a significant reduction in the number and size of organelles.[60] It is assumed that an increase in the autophagic process occurs during this transition from osteoblasts to osteocytes. This increase is due to the need for faster organelle recycling and preservation of nutrients, while the cell develops its actin-rich prolongations, and adjusts to an environment more susceptible to hypoxia.[59] In fact, the expression of LC3 in osteocytes is greater than in osteoblasts.[61]

Osteocytes are long-lived cells located in gaps delimited by mineralized bone matrix and, consequently, are positioned in an environment more susceptible to hypoxia and to the accumulation of oxidative stress.[55] It is believed that under these conditions autophagy plays an important role in the survival of osteocytes.[59] Osteocyte-like cells have increased autophagic activity after undergoing nutrient deprivation and hypoxia in vitro, conditions that are similar to those found in osteocytes in vivo. In addition, in

response to calcium-mediated stress, a hypoxia-inducing transcription factor (HIF-1) positively regulates autophagy, indicating that low oxygen tension serves as a regulator of autophagy in this type of cell.[61] Low-dose glucocorticoid treatment has also been shown to induce autophagy in osteocytes in response to increased oxidative stress caused by treatment, preserving the viability of these cells.[62-64]

Studies indicate that autophagy also plays an important role in bone resorption by osteoclasts.[65] It has been reported that proteins involved in the autophagic pathway are important in the regulation of osteoclastogenesis,[66,67] indicating that this process participates both in bone formation and resorption. There is evidence that resorptive activity is decreased when bafilomycin, an autophagy inhibitor, is added to the culture of osteoclasts.[68,69] In mice, a mutation in the gene encoding the p62 protein has also been reported to result in a phenotype similar to Paget's bone disease, a condition in which there is excessive increase in bone remodeling and susceptibility to fractures.[70] In addition, it has been reported that an increase in autophagy occurs during osteoclastogenesis under conditions of hypoxia and increased oxidative stress caused by glucocorticoid treatment. In this case, it was suggested that autophagy would act as a protective factor reducing cell stress, increasing the formation and viability of osteoclasts.[71-73]

Deletions in genes encoding key proteins in the formation of the autophagosome (ATG5, ATG7, ATG4B and LC3) have been shown to cause changes in the brush border formation of osteoclasts, and, consequently, to reduce bone resorption and increase bone volume, thus preventing bone loss in mice after ovariectomy.[65] Some authors suggest that inhibition of autophagy in osteoclasts may serve as a possible therapeutic mechanism against bone diseases in which there is an excessive increase in bone resorption.[55,72] In fact, it has recently been observed in mice that pharmacological and genetic inhibition of autophagy reduces osteoclastogenesis and bone resorption, inhibiting bone loss caused by ovariectomy or glucocorticoid treatment.[74]

Recently, autophagy has also been pointed as an important factor in the process of bone growth. It was observed that the secretion of type II collagen by chondrocytes of the epiphyseal disc of mice is regulated by autophagy, under the influence of fibroblast growth factor 18 (FGF18).[28] However, the mechanisms by which autophagy regulates the secretion of type II collagen during bone growth are still not fully understood. A clinical trial of gene screening that included 618 adult Chinese subjects demonstrated that the expression of genes regulating the autophagic pathway may influence stature variation in this population.[75] Considering that autophagy increases the viability of chondrocytes, the authors report that this influence can be attributed to the protective role that autophagy exerts on these cells in the epiphyseal disc, thus influencing the growth of long bones.[76]

Other recent studies indicate that the activation of autophagy is also associated with the repair process of bone fractures.[77,78] In this case, it has been proposed that the increase in autophagy that occurs after a bone injury would act as a defense mechanism of the bone cells against cell stress, caused by the sudden reduction or interruption of the nutrient supply, due to bone fracture.[78] Thus, studies indicate that autophagy is a crucial factor for the maintenance of bone tissue homeostasis.

Autophagy, bone loss and osteoporosis: What is the evidence?

It has been widely reported that dysfunction in the autophagic pathway is involved in the development of various pathologies such as: neurodegenerative (e.g. Alzheimer's and Parkinson's)[79] and vascular[80] diseases, cancer,[81] diabetes,[82] obesity,[83] rheumatoid arthritis,[84] and osteoarthritis.[85] Studies suggest that dysregulation of autophagy may also be associated with the process of bone loss and subsequent osteoporosis.[27,86]

Recently, pharmacological inhibition with chloroquine and genetic inhibition by the selective deletion of the *Atg7* gene in monocytes have been shown to reduce osteoclastogenesis and decrease bone loss in animal models.[74,87] Administration of an antibody against sclerostin, an inhibitor of bone formation by osteoblasts, was able to prevent bone loss promoted by treatment with glucocorticoids in male mice.[88] In the study, treatment with high doses of glucocorticoids reduced the percentage of LC3-positive osteoblasts, an autophagic marker, thus reducing the viability of these cells and causing bone loss. Treatment with anti-sclerostin antibody, in turn, maintained the viability of osteoblasts by increasing autophagy in these cells and reducing bone loss caused by glucocorticoid treatment.[88] However, further studies are needed to understand the specific mechanisms by which sclerostin, or its antibody, regulates the autophagic pathway in bone cells.

Such findings indicate that autophagy contributes to the maintenance of bone mass, in part by maintaining the viability of osteoblasts. According to this idea, a deletion of the *FIP200* gene (essential for autophagosome formation) in osteoblasts has been reported to cause osteopenia in rats due to the decrease in bone formation by these cells.[89]

In osteocytes, the deletion of the *Atg7* gene (essential for autophagy initiation) has been shown to promote

bone mass decrease in 6-month-old male and female mice, similar to that caused by natural aging.[86] In another study, a relationship was observed between decreased autophagic activity in osteocytes and bone loss during aging in senile rat models.[29] Recently, this result has also been achieved in senile rat models in which rapamycin, an autophagy inducer, reduced osteoporosis by activating autophagy in osteocytes.[30]

These studies indicate that the mechanism by which autophagy reduces the bone loss caused by aging in these models seems to be related to the antioxidant effect of autophagy on bone cells. The fact that oxidative stress was higher in knockout animal models for *Atg7* reinforces this hypothesis.[86] Also, although estrogen deficiency is considered a major cause of bone loss and osteoporosis in postmenopausal women, studies have shown that increased oxidative stress in bone tissue is one of the major contributing factors to bone loss caused by aging.[59,90,91]

In short, the reduction of autophagy appears to promote increased oxidative stress, causing bone loss, whereas an increase in the autophagic pathway inhibits this effect.[29,30,86] According to this hypothesis, a significant inverse correlation was observed between the level of autophagy in osteocytes and the oxidative stress and bone loss caused by estrogen deficiency in the tibia of ovariectomized rats.[92]

Most evidence indicating a possible relationship between autophagic dysfunction and osteoporosis arises from studies in animal and in vitro models. In humans, however, there is still very little evidence to indicate such a relationship. A genetic screening study in humans revealed that, among the different pathways studied, the expression of autophagic pathway regulatory genes was the only one that showed a direct relationship with the variation in bone mineral density in the distal portion of the radius in 984 individuals.[93] The authors concluded that this relationship may indicate an important participation of the autophagic pathway in the development of osteoporosis.

The preclinical in vivo and in vitro studies included in our review indicate a relationship between the dysregulation of the autophagic pathway and osteoporosis. This possible relationship is summarized in Figure 2.

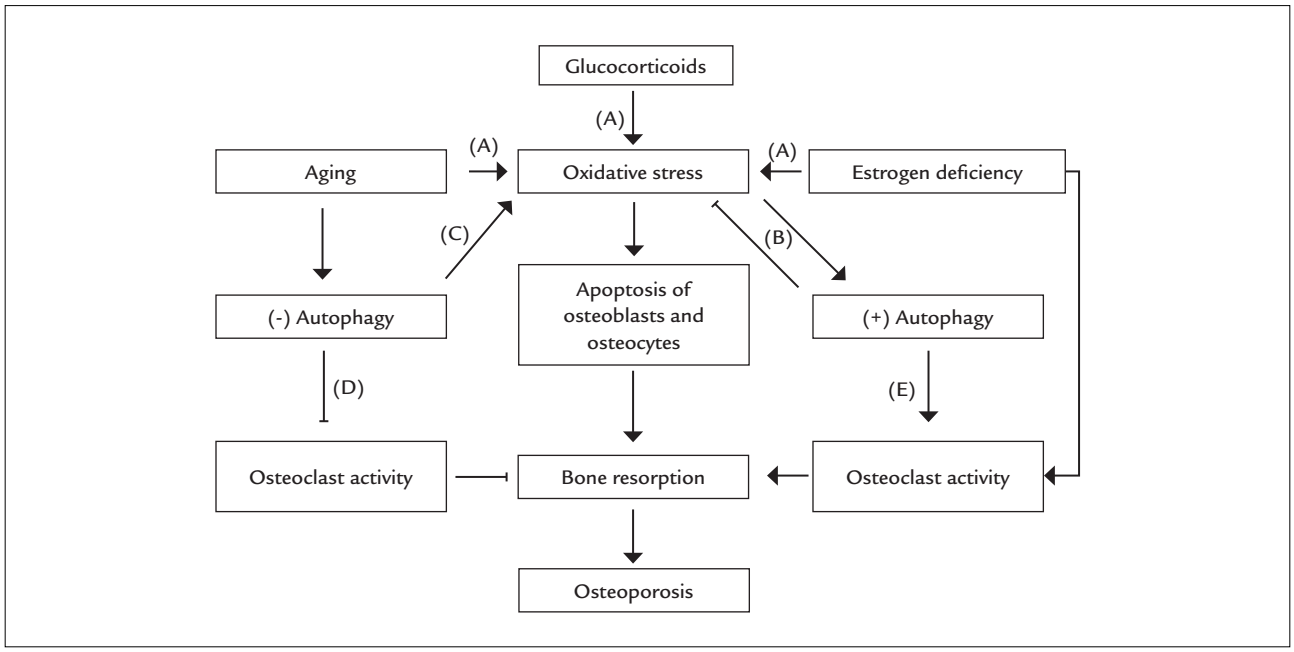

FIGURE 2 Diagram illustrating the possible relationships between autophagy and osteoporosis. (A) Aging, prolonged treatment with glucocorticoids and estrogen deficiency increase oxidative stress in bone tissue, inducing apoptosis of osteoblasts and osteocytes and increased bone resorption by osteoclasts, which induces bone loss and osteoporosis. (B) On the other hand, increased oxidative stress stimulates the autophagic (+) pathway, which reduces the deleterious effect of stress by degrading damaged proteins and organelles. Reduction of cell stress inhibits the apoptosis of osteoblasts and osteocytes, decreasing bone loss and osteoporosis. A reduction of autophagy (-) caused by aging results in increased oxidative stress (C), which can cause bone loss and subsequent osteoporosis. Autophagy is also essential for the viability of osteoclasts. Thus, while the reduction of autophagy decreases the formation and resorptive activity of these cells (D), the increase of autophagy stimulates these processes (E). Arrows indicate stimulatory action and bars indicate inhibitory action.

Conclusion

In conclusion, based on experimental in vivo and in vitro studies, there is a number of evidences that reinforce the existence of a correlation between the autophagy process and osteoporosis. Nevertheless, future studies, especially clinical trials, are needed to confirm the possible relationship between autophagic dysfunction and osteoporosis in humans, as well as to develop future therapies for the prevention and/or treatment of osteoporosis.

Resumo

Osteoporose e autofagia: qual é a relação?

A autofagia é uma via de sobrevivência celular pela qual proteínas e organelas não funcionais são degradadas nos lisossomos, para reciclagem e geração de energia. Assim, a autofagia é fundamental para a manutenção da homeostase e viabilidade da célula, agindo como um controle de qualidade que evita o acúmulo de estruturas desnecessárias e o estresse oxidativo. Um número crescente de estudos tem demonstrado que disfunções na via autofágica estão relacionadas ao surgimento de diversas doenças, como as neurodegenerativas e o câncer. Estudos também têm indicado que a autofagia exerce um importante papel para a manutenção da homeostase óssea; por exemplo, estudos *in vitro* e em animais e humanos mostram que disfunções da autofagia nas células ósseas estão associadas ao surgimento de doenças ósseas, como a osteoporose. Nesta revisão, foram abordados esses estudos, a fim de melhor esclarecer se há uma relação entre disfunção autofágica e osteoporose.

Palavras-chave: autofagia, tecido ósseo, osteoblasto, osteócito, osteoclasto, osteoporose.

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