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## **Autophagy and bone diseases**

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## **Abstract**

Autophagy is a ubiquitous cellular process, allowing the removal and recycling of damaged proteins and organelles. At the basal level, this process plays a role in quality control, thus participating in cellular homeostasis. Autophagy can also be induced by various stresses, such as nutrient deprivation or hypoxia, to allow the cell to survive until conditions improve. In recent years, the role of this process has been widely studied in many pathologies such as neurodegenerative diseases or cancers. In bone tissue, various studies have shown that autophagy is involved in the survival, differentiation and activity of osteoblasts, osteocytes and osteoclasts. The evolution of this knowledge has led to the identification of new molecular pathophysiological mechanisms in bone pathologies.

This review reports the current state of knowledge on the role of autophagy in 4 bone diseases: osteoporosis, which seems to be associated with a decrease in autophagy, osteopetrosis and Paget's disease where the course of the autophagic process is disturbed, and finally osteosarcoma where autophagy seems to play a protumoral role. A better understanding of the involvement of autophagy in these pathologies should eventually lead to the identification of new potential therapeutic targets.

Keywords : Autophagy - Bone – Paget's disease of bone - Osteoporosis - Osteopetrosis - Osteosarcoma

## **1. Autophagy**

### *1.1 Autophagy mechanism*

Macroautophagy, commonly referred to as autophagy, is a ubiquitous cellular mechanism for the removal and recycling of damaged proteins and organelles [1]. At the basal level, this mechanism plays a key role in cellular quality control and homeostasis. Autophagy can also be induced in response to various stresses such as nutrient deprivation, oxidative stress, or hypoxia, to allow cell survival. Finally, under certain conditions, excessive autophagy can lead to cell death [1]. From a molecular point of view, in the presence of nutrients, autophagy is negatively regulated by the mTOR (mammalian target of rapamycin) complex. When the cell is deprived of nutrients or in any other situation of cellular stress, mTOR is inactivated. This inactivation leads to the activation of Unc-51 like autophagy activating kinase (ULK1) and then to the recruitment of a multiprotein complex called PI3K class III complex, which induces autophagy [1]. The autophagic process begins in the cytoplasm, where, under the combined action of different autophagy-related genes (ATGs), a double membrane called phagophore is formed. This phagophore encircles the damaged proteins and organelles and then closes, forming a double-membrane vesicle called an autophagosome. During this process, a cytosolic protein called ATG8 or LC3-I is lipidated and inserted into the double membrane of the autophagosome as LC3-II. Thus LC3-II is considered a marker of autophagosomes. The cellular material to be degraded is sequestered in the autophagosome by the action of specific autophagy receptors including the SQSTM1/p62 (p62) protein, which is considered a substrate of autophagy [2]. Subsequently, the lysosome fuses with the autophagosome, forming an autolysosome. The action of the lysosomal V-ATPase then leads to acidification of the vesicle resulting in degradation and subsequent recycling of material (Figure 1). Autophagy is a dynamic process, in which autophagosomes are constantly formed and then degraded, and is therefore referred to as autophagic flux. Thus, a decrease in LC3-II expression combined with an increase in p62 expression generally reflects a decrease in autophagy. On the other hand, an increase in LC3-II may reflect either

an induction of autophagy or an inhibition of autophagosome maturation, due for example to a defect in lysosomal activity.

The aim of this review is to summarize the main results concerning the role of autophagy in bone tissue and bone pathologies.

## 1.2 Autophagy and bone tissue

The role of autophagy in bone tissue was still unknown ten years ago [2]. However, the observation that most of the bone-promoting molecules such as vitamin D, estradiol, or bisphosphonates were known as autophagy inducers, suggested an important function of this process for bone quality [3]. In the last decade, our understanding of the autophagy role in bone tissue has significantly increased. It has been shown that autophagy is involved in the survival and differentiation of osteoblasts (OB) and osteocytes (OST) [4], and that this process is required for bone mineralization [5]. With regard to osteoclasts (OC), autophagy is involved in their differentiation [6] and some autophagy proteins are required for bone resorption, notably through their involvement in the brush border establishment and in the secretory activity of OCs [7].

## 2. Autophagy role in bone pathologies

### 2.1 Osteoporosis

Osteoporosis (OP) is a diffuse skeletal pathology characterized by major bone fragility that affects one in 3 women after menopause and one in 6 men after 50 years of age [8]. Multiple molecular mechanisms are involved in postmenopausal OP, but two crucial factors for disease onset are the decrease in estrogen and the increase in oxidative stress [9, 10]. Interestingly, estrogen appears to induce autophagy in bone cells [11, 12] while oxidative stress is generated by autophagy deficiency. In addition, a pan-genomic link has been observed between autophagy genes, human height and OP, suggesting that autophagy is involved in this pathology [13, 14]. In addition, several studies also support a role for autophagy in OP: (i) OB and OST from aged mice exhibit a decreased autophagy [15, 16]; (ii) autophagy increases in OST after ovariectomy, likely counterbalancing estrogen loss and

oxidative stress [16, 17]; (iii) autophagy activation in OST through treatment with the mTOR inhibitor rapamycin reduces OP severity [18]; (iv) bone loss related with aging and estrogen deprivation is exacerbated by an autophagy deficiency in OB [16]. All these results show that autophagy induction in OB and OST can compensate the deleterious effects of estrogen drop in OP, suggesting that this pathway represents a promising target for this pathology.

A link between deficient hematopoietic autophagy and OP has recently been evidenced in humans [19]. Moreover, a drastic bone loss along with an imbalance in OST homeostasis was observed in mice after *Atg7* autophagy gene inactivation in hematopoietic cells [19]. This phenotype was associated with the disappearance of H-vessels, connecting OST and hematopoietic cells. In summary, autophagy induction in hematopoietic cells or in OB/OST could represent a new path in OP treatment (Figure 2A).

Some molecules already commercially available are known to modulate autophagy. For example, everolimus, an immunosuppressant that induces autophagy by inhibiting mTOR, decreases bone loss in a postmenopausal osteoporosis model in mice [20]. In humans, coupling an autophagy inducer such as everolimus or resveratrol to a compound with bone tropism could be used to stimulate autophagy specifically in the bone microenvironment.

In corticoid-induced OP, in addition to the decrease in bone formation that is the main mechanism involved in the pathology, there is an increase in resorption related to a prolongation of OC life [21, 22]. Although a relationship between autophagy and glucocorticoid treatment has already been established [23], the underlying molecular mechanism has only very recently been identified, and involves the PI3K/Akt/mTOR autophagic signaling pathway in primary OCs [24]. Thus, both in vitro and in vivo, glucocorticoid-treated OCs display an increased autophagic activity, suggesting that this pathway could be therapeutic target for corticoid-induced OP.

## 2.2 Osteopetrosis

This heterogeneous genetically inherited bone pathology, also called “marble bone disease”, results from OC function alteration. Its severity ranges from mild to a lethal form such as infantile malignant osteopetrosis (IMO) also called autosomal recessive osteopetrosis. This last form of osteopetrosis is characterized by an increased bone mass due to the OC resorption defect. Without bone marrow transplantation, which remains the only treatment to date, this disease is lethal in children [25].

The TCIRG1 gene, encoding the  $\alpha 3$  subunit of V-ATPase, displays the large majority of mutations described in IMO patients although some mutations are also found in the CLC-7 gene or OSTM1 [26, 27]. The resorption activity in OC is affected by these mutations through lysosomal dysfunction. Several autophagy proteins have been shown to participate in the relocalization of this organelle to the brush border where the resorption takes place [7]. Moreover, lysosomes also play a major role in the final step of autophagy, allowing degradation and recycling of autophagosome content. Thus, autophagy appears to play a complex role in this rare bone pathology.

A defect in autophagy, characterized by a reduced LC3-II expression and an increased in p62 protein level, has been observed in OC of the osteopetrotic oc/oc mouse, a mouse model of IMO, carrying a homozygous mutation in the TCIRG1 gene [28]. In addition, mice models carrying OSTM1 or CLC-7 mutations exhibit, on top of an osteoporotic phenotype, a rapid neurodegeneration with an increased LC3-II expression in neurons [29, 30], suggesting a disrupted autophagic flux.

Finally, the PLEKHM1 gene, which is implicated in an intermediate type of human osteopetrosis and in osteoclastic vesicular trafficking in the incisorless rat model [31, 32], plays also a role in the regulation of the endosomal/autophagic pathway through the binding to LC3 and Rab7/HOPS proteins [33, 34]. In summary, most of osteopetrosis gene mutations affecting OC endosomal and lysosomal trafficking, appear to be also associated with neuronal and OC autophagy regulation (Figure 2B).

### *2.3 Paget disease of bone*

Disorganized bone formation as well as an elevated bone resorption are the characteristics of Paget's disease of bone (PDB). Although most patients affected by this chronic focal disease are asymptomatic, joint or neurological complications can be observed due to fissures, fractures, and bone deformities. An increase of bone remodeling is observed, associated with the presence of "hyperactive" giant OCs with many nuclei [35]. Inclusion bodies composed of aggregates of ubiquitinated or misfolded proteins are also present in Pagetic OCs [36]. It seems that environmental and genetic factors are involved in PDB onset. Mutations in the p62/SQSTM1 gene are observed in 40% of familial cases and 10% of sporadic cases. Besides its role as an autophagy receptor, p62 has a key function in NF- $\kappa$ B signaling and apoptosis during osteoclastogenesis [37]. The ubiquitin-associated domain (UBA) of the p62 gene

is the main target of mutations [37] and a point mutation in this domain appears sufficient to trigger PDB-like pathology in mice in which OC exhibit an increased autophagy level [38]. Nevertheless, a study on OC derived from PDB patients suggests an autophagy flux inhibition combined with a build-up of undegraded autophagosomes [39], potentially explaining the presence of inclusion bodies. The study of Usategui-Martín et al. strengthens the previous results as transfection of a p62 gene carrying one of the PDB mutations identified in patients results in autophagosome maturation inhibition [40]. An inhibition of cell proliferation via the NFκ-B pathway was also associated with this mutation [40]. In summary, these experimental results suggest an autophagy deregulation in PDB, which is further supported by ATG gene polymorphisms association with this pathology [41] and the implication of optineurin (OPTN), another autophagy receptor, as a predisposing gene for this disease [42]. More recently, the OATL1 gene, implicated in autophagosome maturation [43], appears to be also involved in PDB [37]. Altogether, these various studies, associated with the role of autophagy/ATG proteins in OC differentiation and function, strongly support the hypothesis of autophagy involvement in PDB (Figure 2C).

#### 2.4 Osteosarcoma

Osteosarcoma (OS) is a malignant bone tumor, primarily of osteoblastic origin, with a peak incidence in childhood and adolescence. The lungs are the most common site of metastasis and the 5-year survival of patients with metastases is approximately 20%. From a genetic perspective, OS has major genomic complexity. Among the tumor suppressor genes frequently inactivated in OS, some such as RB1, PTEN or TP53 regulate autophagy. In parallel, various oncogenes that can be activated in OS also regulate autophagy. These data suggest a deregulation of autophagy in OS [44].

A dual role of autophagy is observed in OS as in other tumor types. OS tumor cells use autophagy as a survival pathway for treatment resistance, proliferation and cancer stem cell protection [44]. Thus, it seems interesting to specifically block autophagy in these cells, in order to potentiate the efficacy of classical therapies. For this purpose, we dispose of autophagy inhibiting molecules, such as hydroxychloroquine (late inhibitor) or VPS34 inhibitors (early inhibitors). Numerous clinical trials are underway, combining conventional chemotherapies with autophagy modulators [45]. However,



autophagy can also lead to cancer cell death, although the conditions required to transform pro-survival autophagy into a death process are not yet elucidated [44].

### **3. Conclusion**

Over the past decade, our knowledge regarding the role of autophagy in bone tissue and bone pathology has significantly increased. Modulation of autophagy has emerged as an exciting new avenue of research for the development of new therapeutic strategies.

In postmenopausal OP, the body of preclinical work in animal models suggests that a decrease in autophagy in OB/OST participates in the development of this pathology, although definitive proof in humans remains to be established. Understanding the reason for this age-related decrease remains an important issue as these data could help identify new therapeutic molecules. In this context, an age-related increase in the expression of Rubicon, a negative regulator of autophagy, has been observed in different tissues in mice. Although the age-dependent expression level of Rubicon has not yet been studied in bone, it has already been shown that inactivation of this gene restores autophagy, thus reducing age-related phenotypes [46]. Thus, stimulating autophagy in the bone microenvironment appears to be a promising strategy for the treatment of OP. In osteopetrosis and PDB, the autophagic process is disturbed either indirectly by mutations affecting lysosomal function (osteopetrosis) or directly by mutations affecting autophagy receptors (PDB). In both cases, it is likely that autophagy perturbation contributes to the pathology by affecting osteoclastic function.

Finally, in osteosarcoma as in other tumor types, the role of autophagy is complex but tumor cells generally use this process to resist the multiple stresses to which they are subjected. In addition to blocking autophagy, identifying the molecular mechanisms that allow switching from pro-survival autophagy to cell death could lead to the identification of new treatment pathways.

In conclusion, we are at the beginning of deciphering the role of autophagy in bone tissue and bone pathology. Continued studies in this area offer the prospect of new therapeutic solutions in a wide spectrum of pathologies for which options are still sometimes limited.

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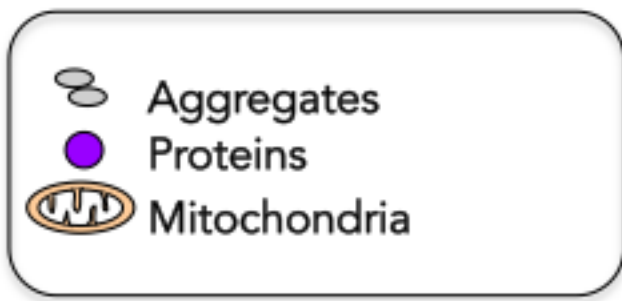
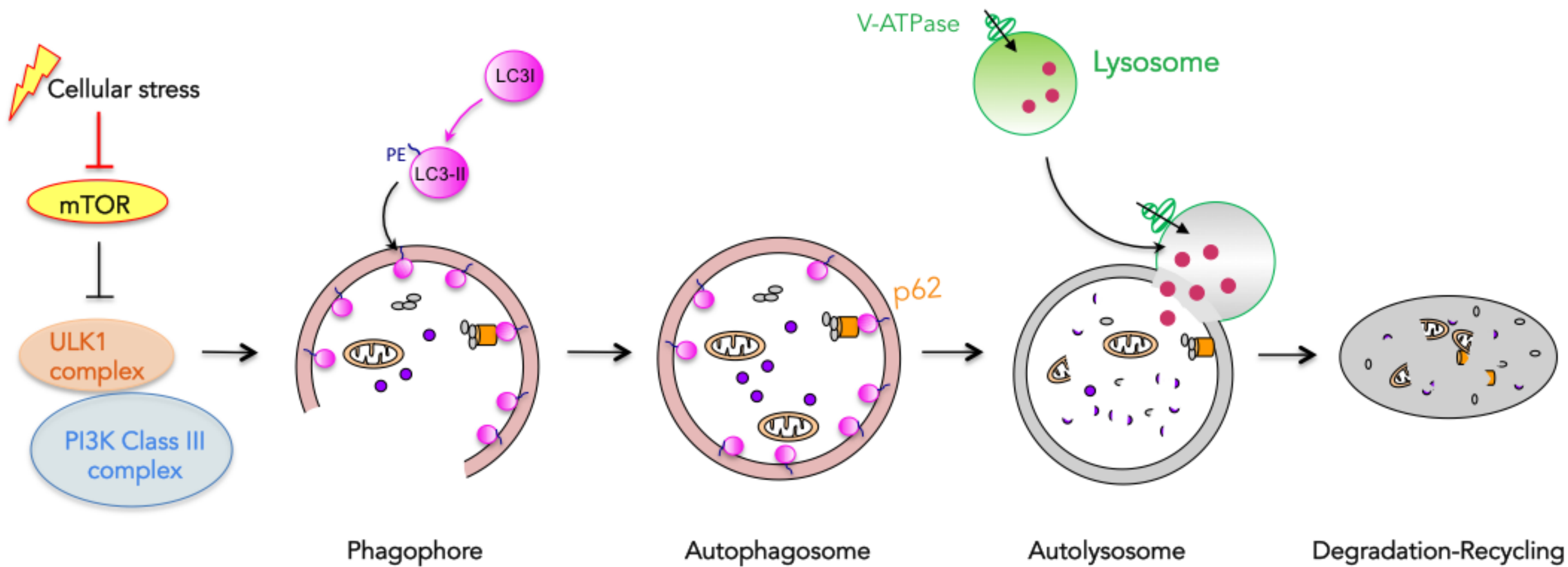
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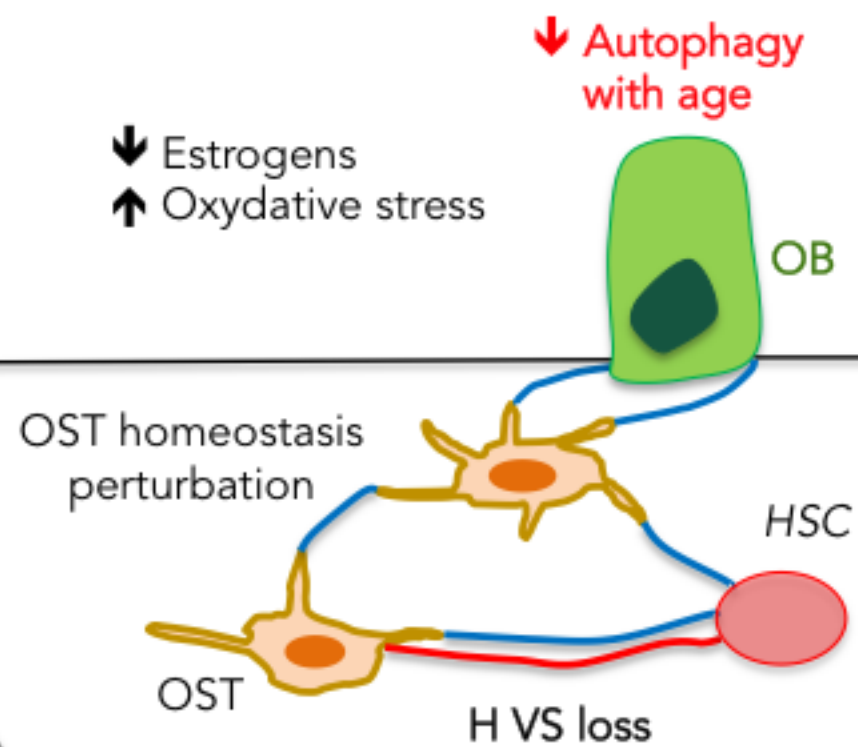
**Figure 1: Autophagy mechanism.** The mechanism of autophagy and the main molecular complexes involved are presented. In response to cellular stress, mTOR is inactivated. Autophagy is then triggered by the action of the ULK1 complex and the PI3K class III complex. A double membrane called phagophore is formed in the cytosol, and encloses damaged organelles, aggregates and proteins. The phagophore then closes, leading to the formation of an autophagosome. During this process, a cytosolic protein named LC3-I is lipidated and inserted into the double membrane as LC3-II. The p62 protein acts as a receptor for autophagy by binding the ubiquitinated proteins on the one hand and the LC3-II protein on the other. Finally, the autophagosome fuses with the lysosome to degrade, thanks to the action of V-ATPase, the contents which will be recycled. PE : Phosphatidylethanolamine.

**Figure 2: Autophagy in bone pathologies.** (A) Development of osteoporosis is associated with decreased autophagy with age in OB, OST, and hematopoietic stem cells (HSCs). (B) Mutations identified in osteopetrosis (OMI) induce an autophagy defect or an autophagic flux disturbance in OC, resulting in decreased resorption. (C) In Paget's disease of bone, mutations in p62, or OPTN could promote inhibition of autophagic flux and the appearance of inclusion bodies. This autophagy defect could be involved in the development of PDB, especially through the role of autophagy and ATG proteins in the differentiation and function of OC. VS: vessels.



A

## Osteoporosis



B

## Osteopetrosis

Mutations *TCIRG1*,  
*CLC7*, *OSTM1*

Defective autophagy /  
Flux perturbation

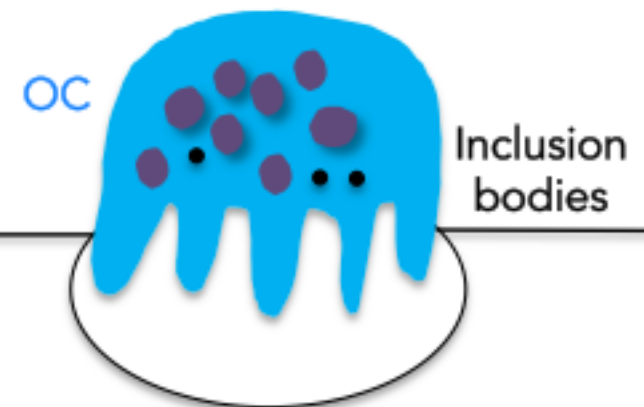


Inefficient OC  
↓ Resorption

C

## Paget's disease of bone

p62 mutations  
Flux inhibition

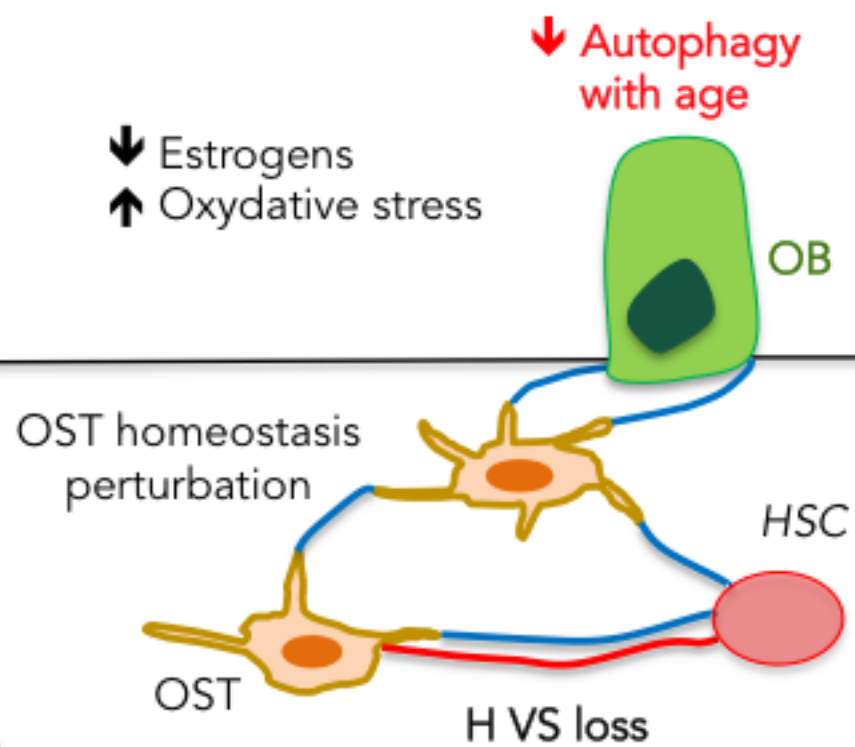


Overactive OC  
↑ Resorption



A

### Osteoporosis



B

### Osteopetrosis

Mutations *TCIRG1*,  
*CLC7*, *OSTM1*

Defective autophagy /  
Flux perturbation

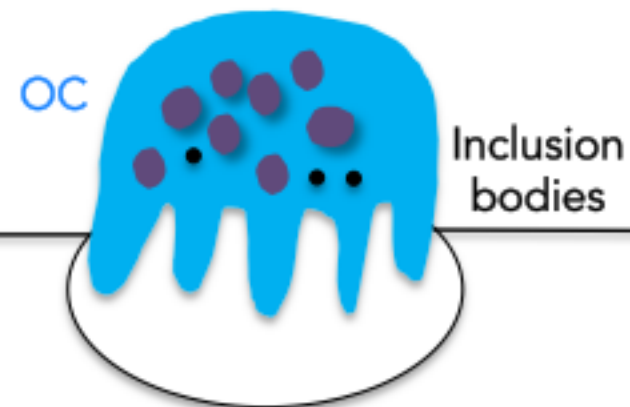


Inefficient OC  
↓ Resorption

C

### Paget's disease of bone

p62 mutations  
Flux inhibition



Overactive OC  
↑ Resorption