ORGANELLE
REVIEW | OPEN ACCESS

Wang *et al.* Organelle (2025) 3:3
https://doi.org/10.61747/0ifp.202503003

Organelles and Extracellular Vesicles in Bone Aging

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Received: 9 January 2025; Revised: 17 March 2025; Accepted: 11 April 2025

Journal homepage: https://www.organelles.org

ABSTRACT

Bone aging is characterized by a decline in bone mass and structural integrity, increasing fracture and osteoporosis risk in elderly. This process involves cellular and molecular changes, including impaired osteoblast and osteoclast activity, reduced osteocyte viability, and disrupted intercellular communication within the bone microenvironment. Organelles such as mitochondria, endoplasmic reticulum (ER), lysosomes, and the Golgi apparatus play crucial roles in maintaining cellular homeostasis. During aging, mitochondria show reduced ATP production and increased reactive oxygen species, leading to oxidative stress and impaired bone cell function. ER stress causes accumulation of misfolded proteins, affecting cell survival. Lysosomal dysfunction hampers autophagy, resulting in damaged protein and organelle accumulation. Golgi impairments disrupt protein modification and secretion essential for bone matrix formation. Extracellular vesicles (EVs), including exosomes and microvesicles, mediate intercellular communication in the bone microenvironment by transferring bioactive molecules that influence osteogenesis and osteoclastogenesis. Organelle dysfunctions alter EVs biogenesis and cargo. These changes propagate stress signals and modulate inflammatory responses, which in turn exacerbate bone degeneration. This mini-review summarizes the age-related functional alterations of key organelles in bone cells and elucidates their interactions with EVs. Understanding these relationships provides insights into bone aging pathogenesis and aids in developing therapeutic strategies. Targeting organelle function and modulating EV-mediated communication offer promising approaches to delay or reverse bone aging, improving skeletal health in the aging population.

Key words: Organelles; Extracellular vesicles; Bone aging; Bone metabolism

1. INTRODUCTION

Bone health is fundamental to maintaining the body's mobility and metabolic balance. As the global population ages, osteoporosis and related fractures have become significant public health concerns, leading to increased morbidity, mortality, and healthcare costs among the elderly [1,2]. Osteoporosis is a global health crisis, affecting 200 million women worldwide [3]. Half of postmenopausal women will experience an osteoporotic fracture, with vertebral fractures significantly impairing mobility and increasing mortality by 2.8 times. As the population ages, hip fracture rates are expected to rise by 310% by 2050. Osteoporosis-related fractures cost Europe over €56 billion annually, with hip fractures alone averaging $38,000 in care costs [4]. The fracture risk in postmenopausal women exceeds the combined risk of breast, ovarian, and uterine cancers, emphasizing its critical impact on women's health. These statistics highlight osteoporosis as a "silent epidemic" and set the stage for intervention strategies. Bone aging is characterized by a progressive loss of bone mass and deterioration of bone microarchitecture, resulting from an imbalance between bone formation and resorption [5]. Understanding the underlying mechanisms of bone aging is crucial for developing effective prevention and treatment strategies.

At the cellular level, bone aging involves complex pathophysiological changes, including dysfunctions of various organelles such as mitochondria, endoplasmic reticulum (ER), lysosomes, and the Golgi apparatus [6]. These organelles are critical for maintaining cellular homeostasis, regulating processes like energy production, protein synthesis and folding, waste degradation, and intracellular trafficking [7]. Their dysfunction can lead to impaired energy metabolism, increased oxidative stress, accumulation of misfolded proteins, and disrupted autophagy—all contributing to the decline in bone cell function. Oxidative stress plays a crucial role in linking cellular senescence to bone aging through its complex effects on extracellular vesicles (EVs) dynamics and organelle homeostasis. During aging, the accumulation of reactive oxygen species (ROS) induces covalent modifications to essential biomolecules, including lipid peroxidation, protein carbonylation, and DNA oxidation, which fundamentally alter EVs biogenesis and cargo selection [8]. These redox-induced changes occur through three interconnected mechanisms. First, ROS activation of NF-κB signaling triggers pro-inflammatory cascades that disrupt bone remodeling by modulating osteoclast-osteoblast communication

The Article Processing Charge for this work was funded by the authors.

via EV-mediated signaling. Second, oxidative damage impairs the endosomal sorting complexes required for transport (ESCRT), compromising multivesicular body integrity and cargo sorting, resulting in EVs with aberrant molecular profiles [9]. Lastly, mitochondrial and endoplasmic reticulum dysfunction under oxidative conditions disrupts EVs production at the source while facilitating the packaging of organelle-derived damage-associated molecular patterns (DAMPs) into EVs [10]. These modified vesicles then propagate oxidative stress signals across bone tissue microenvironments. This tripartite mechanism forms a self-perpetuating cycle in which oxidative stress both generates and amplifies dysfunctional EVs, contributing to the progressive deterioration of bone quality with aging [11]. The integration of these pathways emphasizes the importance of targeting organelle-vesicle networks in developing therapeutic strategies to combat skeletal aging. Concurrently, EVs, including exosomes and microvesicles, have emerged as key mediators of intercellular communication within the bone microenvironment. Changes in the biogenesis, cargo, and secretion of EVs during aging can influence bone remodeling processes by affecting osteoblasts, osteoclasts, and osteocytes (Figure 1).

Given the intricate interplay between organelle dysfunction and EV-mediated signaling in bone aging, it is essential to elucidate these mechanisms to identify novel therapeutic targets. This mini-review aims to provide a comprehensive overview of the roles of organelles and EVs in the process of bone aging. By summarizing current knowledge and highlighting recent advances, we seek to enhance the understanding of bone aging pathogenesis and underscore the potential of targeting organelle functions and EV-mediated communication in developing effective interventions for age-related bone diseases.

2. MITOCHONDRIAL DYSFUNCTION AND EVS IN BONE AGING

2.1. Mitochondrial Dysfunction

Mitochondria are essential organelles responsible for ATP production through oxidative phosphorylation, regulation of cellular metabolism, and control of apoptosis. Age-related changes in mitochondrial membrane potential have been shown to lead to mitochondrial dysfunction, including increased mitochondrial permeability and leakage. These alterations may further affect cellular homeostasis and contribute to the release of mitochondrial components into EVs, thereby influencing intercellular communication. In bone cells, mitochondrial function is crucial for energy-intensive processes such as bone formation and remodeling. Aging leads to a decline in mitochondrial ATP production due to impairments in the electron transport chain and decreased mitochondrial biogenesis [12,13]. This reduction adversely affects bone marrow mesenchymal stem cells (BMSCs), which require adequate ATP for proliferation and differentiation into osteoblasts. The diminished activity of BMSCs results in decreased

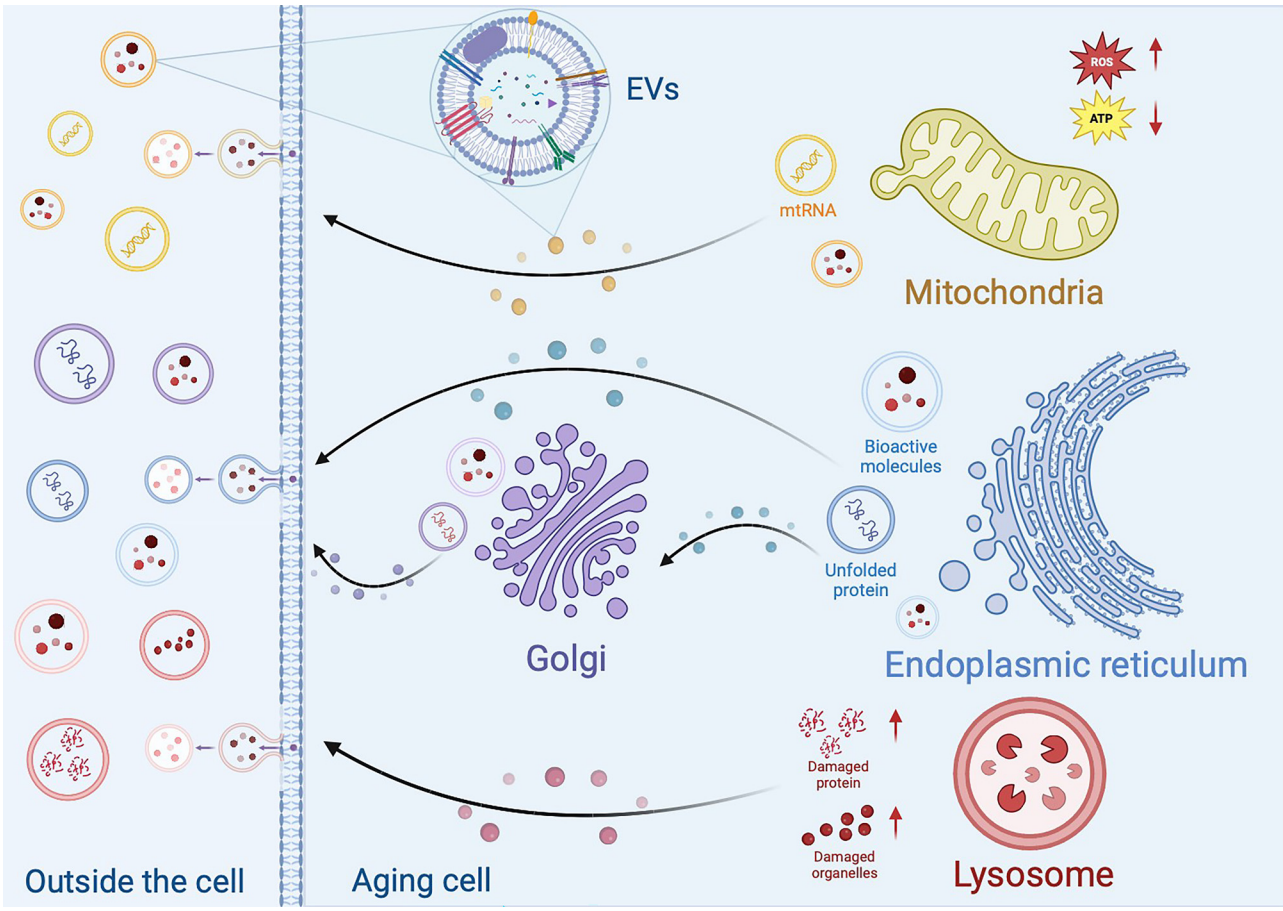

Figure 1. Organelle and EVs in an Aging Cell

Organelle dysfunction in aging cells, including impaired mitochondria, ER, lysosomes, and the Golgi apparatus, disrupts cellular homeostasis. These dysfunctions lead to the release of EVs containing damaged organelle components and stress signals.

osteogenesis and bone formation, contributing to age-related bone loss and increased fracture risk. Additionally, aging cells exhibit increased production of reactive oxygen species (ROS) due to mitochondrial inefficiencies [14]. Elevated ROS levels cause oxidative damage to mitochondrial DNA, proteins, and lipids, further impairing mitochondrial function. In bone cells, excessive ROS induces apoptosis and senescence of osteoblasts and osteocytes while promoting osteoclast differentiation and activity, accelerating bone resorption and compromising bone structural integrity. Furthermore, aging is associated with reduced mitophagy—the process by which cells remove damaged mitochondria—due to decreased expression of key autophagy-related genes and proteins [15]. The accumulation of damaged mitochondria exacerbates oxidative stress and disrupts cellular metabolism, hampering the regenerative capacity of BMSCs and osteoblasts, and contributing to metabolic disorders that negatively affect bone density and strength. During bone cell aging, mitophagy is essential for maintaining cellular homeostasis by selectively removing damaged mitochondria through the PINK1/Parkin pathway. This process helps to preserve energy balance and overall cell health. However, as aging progresses, the PINK1/Parkin signaling pathway becomes disrupted, preventing the effective clearance of damaged mitochondria [16]. This dysfunction leads to an accumulation of defective mitochondria, which triggers oxidative stress. The resulting oxidative stress damages mitochondrial DNA and proteins and accelerates the production of ROS, further impairing bone cell function. Consequently, this contributes to increased bone resorption and a reduced capacity for bone formation [17].

2.2. Relationship Between Mitochondria and EVs

Cells experiencing mitochondrial dysfunction and elevated ROS levels can release EVs containing oxidative stress markers, signaling molecules, and even mitochondrial DNA (mtDNA) [18,19]. These EVs can be taken up by neighboring bone cells, propagating oxidative stress and inducing similar dysfunctions in recipient cells, which amplifies the detrimental effects of oxidative damage within the bone microenvironment and exacerbates bone aging. Extracellular mtDNA acts as a damage-associated molecular pattern (DAMP) that activates innate immune responses through receptors like Toll-like receptor 9 (TLR9). The uptake of mtDNA-containing EVs by bone cells triggers inflammatory pathways, leading to chronic inflammation that promotes osteoclastogenesis and inhibits osteoblast function, further contributing to bone degeneration. Additionally, EVs can carry bioactive molecules such as proteins, microRNAs, and other nucleic acids that influence mitochondrial function in recipient cells [20]. For instance, EVs from healthy cells may transfer mitochondrial components or signaling molecules that enhance mitochondrial biogenesis and oxidative phosphorylation in target bone cells. Conversely, EVs derived from dysfunctional or aged cells may contain inhibitory factors that impair mitochondrial function in recipient cells, disrupting their energy metabolism and viability.

3. ENDOPLASMIC RETICULUM DYSFUNCTION AND EVS IN BONE AGING

3.1. Endoplasmic Reticulum Dysfunction

The endoplasmic reticulum (ER) is essential for protein synthesis, folding, post-translational modifications, lipid metabolism, and calcium storage in bone cells. Proper ER function ensures the production of bone matrix proteins and cellular homeostasis. During aging, the ER's folding capacity can be overwhelmed, leading to the accumulation of misfolded or unfolded proteins and triggering the unfolded protein response (UPR), an adaptive mechanism aimed at restoring ER function. However, prolonged ER stress results in chronic UPR activation, which becomes maladaptive [21]. ER stress contributes to the dysfunction of bone cells. As the folding capacity of the ER declines, unfolded proteins accumulate, triggering the UPR. When the UPR becomes excessively activated, it inhibits the proliferation, differentiation, and survival of bone cells. In aged bone cells, prolonged ER stress intensifies bone resorption and disrupts the synthesis of bone matrix proteins, thereby accelerating degenerative changes in bone tissue. In osteoblasts and osteocytes, persistent UPR impairs proliferation and differentiation, reducing bone formation and contributing to bone loss [21,22]. Sustained UPR can also initiate apoptotic pathways through activation of C/EBP homologous protein (CHOP) and caspase-12, leading to cell death and further diminishing bone-forming capacity. Additionally, ER stress disrupts calcium homeostasis by causing the release of Ca^{2+} into the cytosol due to dysfunction of ER calcium channels and pumps [21]. This imbalance affects calcium-dependent processes, including the synthesis and secretion of bone matrix proteins like collagen and osteocalcin, weakening bone strength by compromising extracellular matrix integrity [23]. Chronic ER stress activates inflammatory signaling pathways, notably the NF-κB pathway, increasing expression of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β [24]. These cytokines create a pro-inflammatory microenvironment within bone tissue, promoting osteoclast differentiation and activity while inhibiting osteoblast function, accelerating bone resorption relative to bone formation, and increasing the risk of osteoporosis.

3.2. Relationship Between Endoplasmic Reticulum and EVs

Cells under ER stress can increase the secretion of EVs to alleviate the accumulation of misfolded or unfolded proteins [25]. These EVs may contain misfolded proteins, molecular chaperones, and stress-related signaling molecules. The efficiency of protein folding decreases, causing an accumulation of misfolded proteins, which triggers the UPR. Prolonged stress disrupts cell function, especially in osteoblasts and osteoclasts, impairing the balance of bone formation and resorption. Increased inflammation and oxidative stress also exacerbate ER stress, further damaging bone cell function. Additionally, age-related mitochondrial dysfunction and reduced autophagy contribute to increased ER stress, ultimately leading to osteoporosis and bone degeneration. While exporting these problematic proteins via EVs helps reduce ER burden, the release into the bone microenvironment can adversely affect neighboring cells. EVs derived from ER-stressed osteoblasts may carry unfolded proteins and stress signals that, when taken up by recipient cells, induce ER stress in those cells as well [26], impairing the function of neighboring osteoblasts and osteocytes and further diminishing bone formation. Additionally, EVs released from ER-stressed cells can carry pro-inflammatory cytokines and microRNAs that modulate inflammatory responses in recipient cells [27]. For example, EVs containing IL-6 mRNA or miRNAs that upregulate pro-inflammatory pathways enhance the inflammatory state of the bone microenvironment [28]. When these EVs are taken up by osteoclast precursors, they promote osteoclastogenesis, increasing bone resorption [29]. The inflammatory signals carried by EVs also inhibit osteoblast differentiation and activity, exacerbating bone degeneration.

4. LYSOSOMAL DYSFUNCTION AND EVS IN BONE AGING

4.1. Lysosomal Dysfunction

Lysosomes are vital organelles responsible for degrading and recycling cellular waste, damaged organelles, and macromolecules through autophagy and other catabolic processes. In bone cells, lysosomal function is crucial for maintaining cellular homeostasis and regulating bone remodeling—a balance between bone formation by osteoblasts and bone resorption by osteoclasts. Aging can impair lysosomal function, leading to disruptions in autophagy and acidification, which have significant implications for bone health. Autophagy is a cellular degradation pathway where lysosomes digest and recycle intracellular components, including misfolded proteins and damaged organelles. In aging, there is a decline in autophagy activity due to reduced expression of autophagy-related genes and impaired lysosomal function [30]. This decrease leads to the accumulation of intracellular waste and damaged organelles in BMSCs, inhibiting their proliferation and differentiation into osteoblasts. The reduced regenerative capacity of BMSCs results in decreased osteogenesis, contributing to bone loss and increased fracture risk in the elderly. In osteoblasts and osteocytes, impaired autophagy can lead to cellular senescence and apoptosis, further exacerbating bone density reduction. In osteoclasts, autophagy is essential for their differentiation and resorptive function; dysfunction can disrupt bone resorption, leading to imbalances in bone remodeling [31]. Several autophagy-related genes, such as ATG5, ATG7, and Beclin-1, play pivotal roles in the regulation of autophagic processes [32]. Dysregulation of these genes can impair mitochondrial quality control and contribute to mitochondrial dysfunction and the release of mitochondrial components into EVs. Additionally, lysosomes maintain an acidic environment (low pH) essential for the optimal activity of hydrolytic enzymes involved in degradation processes. In osteoclasts, the acidification of the resorption lacuna—a specialized compartment between the osteoclast and the bone surface—is crucial for bone resorption [33]. Aging can impair the acidification mechanisms in lysosomes and resorption lacunae due to altered expression or function of proton pumps like the vacuolar H^+-ATPase [34]. A pH imbalance affects the ability of osteoclasts to dissolve the mineralized bone matrix, leading to reduced bone resorption activity. This disruption contributes to an imbalance between bone formation and resorption, potentially resulting in osteosclerosis or ineffective removal of old or damaged bone tissue, compromising bone quality and strength.

4.2. Relationship Between Lysosomes and EVs

When autophagy is blocked or lysosomal function is compromised, cells can activate alternative pathways to eliminate accumulated waste and damaged components. One such pathway involves the increased secretion of EVs [35]. EVs can encapsulate misfolded proteins, damaged organelles, and other waste products, exporting them out of the cell to maintain intracellular homeostasis. In bone cells, increased EV secretion due to impaired autophagy may affect the bone microenvironment. EVs containing waste materials or damage-associated molecular patterns (DAMPs) can be taken up by neighboring cells, potentially inducing stress responses or inflammation that impact bone remodeling processes [36]. This can lead to a propagation of cellular dysfunction and contribute to bone degeneration associated with aging. Additionally, EVs can carry autophagy-related proteins, microRNAs (miRNAs), and other signaling molecules that influence the autophagic function of recipient cells [37]. For example, EVs from healthy cells may contain factors like miR-223 or proteins such as LC3 and Beclin-1 that promote autophagy in target cells, aiding in the maintenance of cellular homeostasis and function. Conversely, EVs derived from cells with lysosomal dysfunction may carry inhibitory signals or stress-inducing molecules that impair autophagy in recipient cells [38]. In the context of bone remodeling, such EV-mediated communication can alter the balance between osteoblast and osteoclast activity. Inhibiting autophagy in osteoblasts can reduce bone formation, while in osteoclasts, it may impair resorption activity, both contributing to skeletal fragility.

5. GOLGI APPARATUS DYSFUNCTION AND EVS IN BONE AGING

5.1. Golgi Apparatus Dysfunction

The Golgi apparatus is an essential organelle involved in the post-translational modification, sorting, and trafficking of proteins and lipids within the cell. Aging causes structural and functional changes in the Golgi apparatus, primarily due to oxidative stress, mitochondrial dysfunction, and the accumulation of misfolded proteins. These disruptions impair the Golgi's essential functions, such as protein glycosylation, sulfation, and sorting, which are critical for the proper processing and secretion of bone matrix proteins. As a result, Golgi dysfunction compromises the modification and secretion of key proteins like collagen and osteocalcin, weakening bone structure. In bone cells, it plays a critical role in processing and secreting bone matrix proteins, including collagen, osteocalcin, and alkaline phosphatase, which are vital for bone mineralization and extracellular matrix formation. Dysfunction of the Golgi apparatus during aging can impair these processes, leading to defective bone matrix metabolism and contributing to skeletal fragility. Aging can lead to structural and functional alterations in the Golgi apparatus, affecting its capacity to modify and secrete proteins properly [39]. Disruptions in glycosylation, sulfation, and phosphorylation processes can result in misfolded or improperly processed bone matrix proteins. Such defects interfere with the bone mineralization process by reducing the availability of functional proteins necessary for extracellular matrix assembly. Consequently, bone strength and integrity are compromised due to the formation of a deficient bone matrix. Additionally, Golgi stress, resulting from dysfunction or overload of the Golgi apparatus, can activate signaling pathways associated with apoptosis and cellular aging [39]. In bone cells, Golgi stress can induce apoptosis through the activation of caspases and pro-apoptotic factors. Increased apoptosis of osteoblasts and osteocytes reduces bone formation and maintenance, contributing to bone loss. Moreover, Golgi apparatus dysfunction may influence the secretion of aging-related signaling molecules, further promoting cellular senescence within the bone microenvironment.

5.2. Relationship Between Golgi Apparatus and EVs

The Golgi apparatus is involved in the biogenesis of certain types of EVs, particularly those derived from the secretory pathway [40]. Golgi dysfunction can alter the sorting and packaging of proteins and lipids into EVs, affecting their composition and function. Changes in the quality of EVs due to Golgi stress can impact intercellular communication by modifying the cargo delivered to recipient cells. This can have downstream effects on bone remodeling processes by influencing the behavior of osteoblasts, osteoclasts, and osteocytes. EVs regulated by the Golgi apparatus play significant roles in the intercellular transport of proteins, lipids, and signaling molecules essential for bone metabolism [40]. Proper functioning of the Golgi ensures

that EVs carry bioactive molecules that promote osteogenesis and regulate bone resorption. Dysregulation of Golgi-mediated protein transport can lead to the secretion of EVs with aberrant or deficient cargo, disrupting the balance of bone remodeling. This may result in impaired bone formation, excessive bone resorption, or both, contributing to bone diseases associated with aging. In addition, aging-related Golgi dysfunction affects the biogenesis of EVs [41]. Since the Golgi is responsible for packaging proteins and lipids into EVs, its dysfunction leads to the secretion of EVs with altered cargo, such as misfolded proteins or incomplete bone matrix components. These aberrant EVs propagate stress signals, exacerbate inflammation, and promote bone resorption while inhibiting osteoblast function, thereby contributing to the progression of bone degeneration.

6. INTERACTIONS BETWEEN ORGANELLES AND SYSTEMIC EFFECTS OF EVS

6.1. Propagation of Organelle Damage Signals

The interplay between different organelles is crucial for maintaining cellular homeostasis. During aging, the dysfunction of one organelle can impact others, leading to a cascade of cellular disturbances. The mitochondria-associated endoplasmic reticulum membranes (MAMs) are specialized domains where the ER and mitochondria are in close contact. MAMs facilitate the exchange of calcium ions (Ca^{2+}) and lipids between these organelles, playing a critical role in calcium homeostasis and energy metabolism [42]. During aging, the function of MAMs decreases, leading to disrupted calcium signaling and impaired mitochondrial function. Reduced MAM activity results in decreased calcium transfer from the ER to mitochondria, affecting mitochondrial ATP production and increasing ROS generation [42]. The accumulation of ROS induces oxidative stress, damaging mitochondrial DNA and proteins, which further impairs mitochondrial function. This dysfunction can trigger the UPR in the ER due to disrupted protein folding environments (Figure 2).

Damaged mitochondria and stressed ER can release DAMPs and stress signals, which are packaged into EVs [43]. These EVs carry proteins, lipids, and microRNAs reflective of organelle dysfunction. When taken up by neighboring bone cells, such as osteoblasts and osteoclasts, these EVs propagate damage signals, inducing ER stress and mitochondrial dysfunction in recipient cells. This amplification of cellular stress contributes to bone degeneration by impairing bone formation and enhancing bone resorption. Additionally, lysosomes and mitochondria interact closely through processes like mitophagy, where damaged mitochondria are selectively degraded to maintain cellular health. Aging-associated autophagy disorders can impair lysosomal function, leading to the accumulation of dysfunctional mitochondria [44]. The reduced clearance of damaged mitochondria exacerbates oxidative stress and disrupts cellular metabolism. Damaged mitochondria can release mtDNA and other DAMPs into the cytosol, which may be encapsulated into EVs and secreted [45].

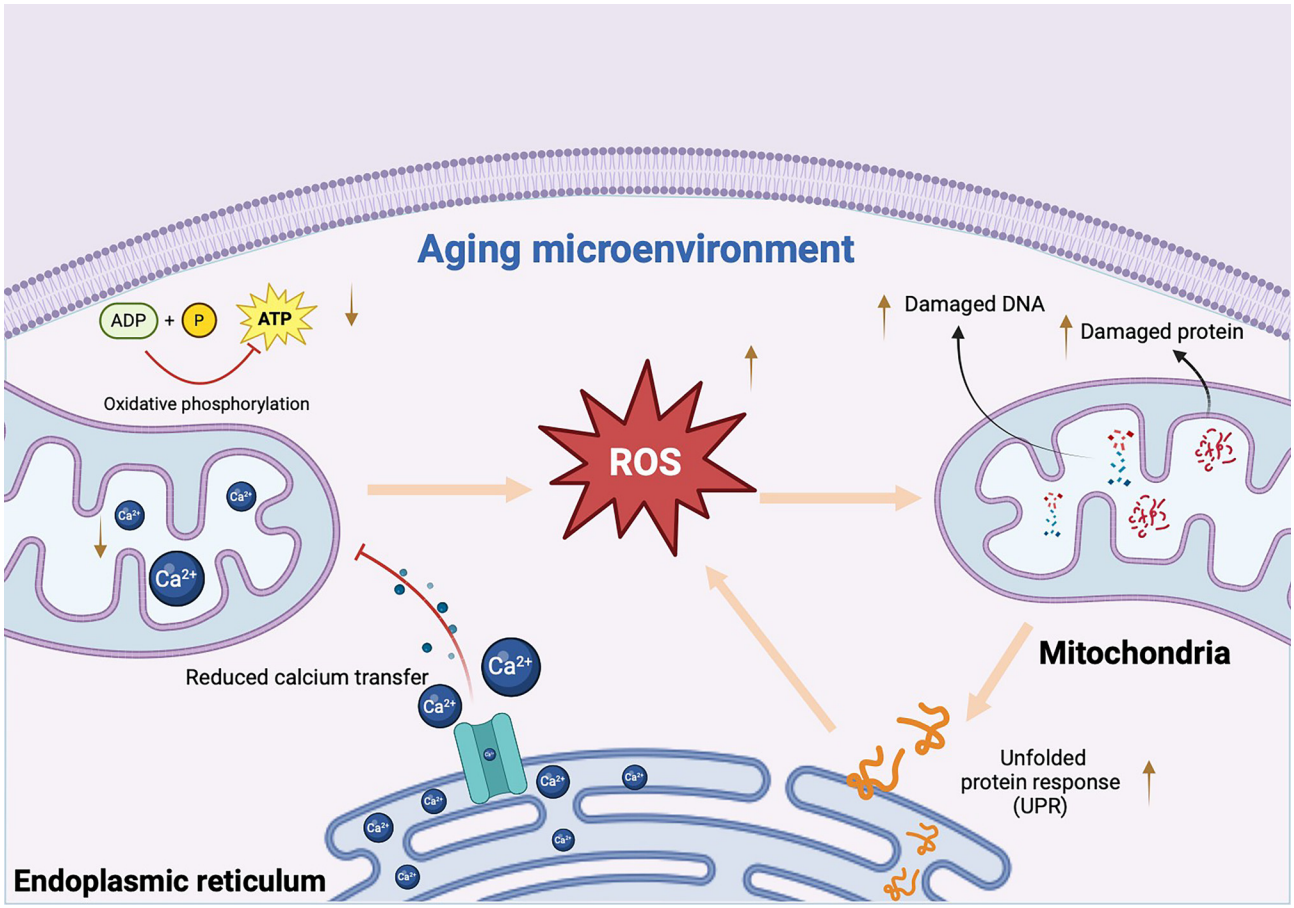

Figure 2.
Aging-induced dysfunction of MAMs disrupts calcium transfer and increases ROS production, damaging mitochondrial DNA and proteins. This triggers ER stress and UPR, leading to the release of stress signals and DAMPs in EVs.

These EVs carrying mitochondrial components can be internalized by surrounding bone cells, triggering inflammatory responses and mitochondrial dysfunction in recipient cells. The intercellular transfer of mitochondrial damage via EVs promotes a spread of cellular dysfunction within the bone microenvironment, contributing to bone loss and structural deterioration.

6.2. Systemic Propagation of EVs in Bone Aging

As cells age, they undergo functional declines and may enter a state of senescence, characterized by the secretion of various bioactive molecules collectively known as the senescence-associated secretory phenotype (SASP) [46]. Senescent cells release EVs enriched with aging-related molecules, including pro-inflammatory cytokines, growth factors, and microRNAs that modulate gene expression in recipient cells. These EVs can travel beyond the local bone environment through the circulatory system, affecting distant cells and tissues [47], (Figure 3). In the context of bone aging, EVs from aged or senescent bone cells can impair the function of BMSCs, osteoblasts, and osteoclasts in remote sites. By altering cellular proliferation, differentiation, and activity, these EVs contribute to systemic bone loss and increased susceptibility to fractures.

Moreover, chronic, low-grade inflammation, or "inflammaging," is a hallmark of aging and plays a significant role in age-related bone diseases [43]. EVs are key mediators in maintaining this inflammatory microenvironment within bone tissue. Stressed or damaged bone cells release EVs carrying pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) [43]. When these EVs are

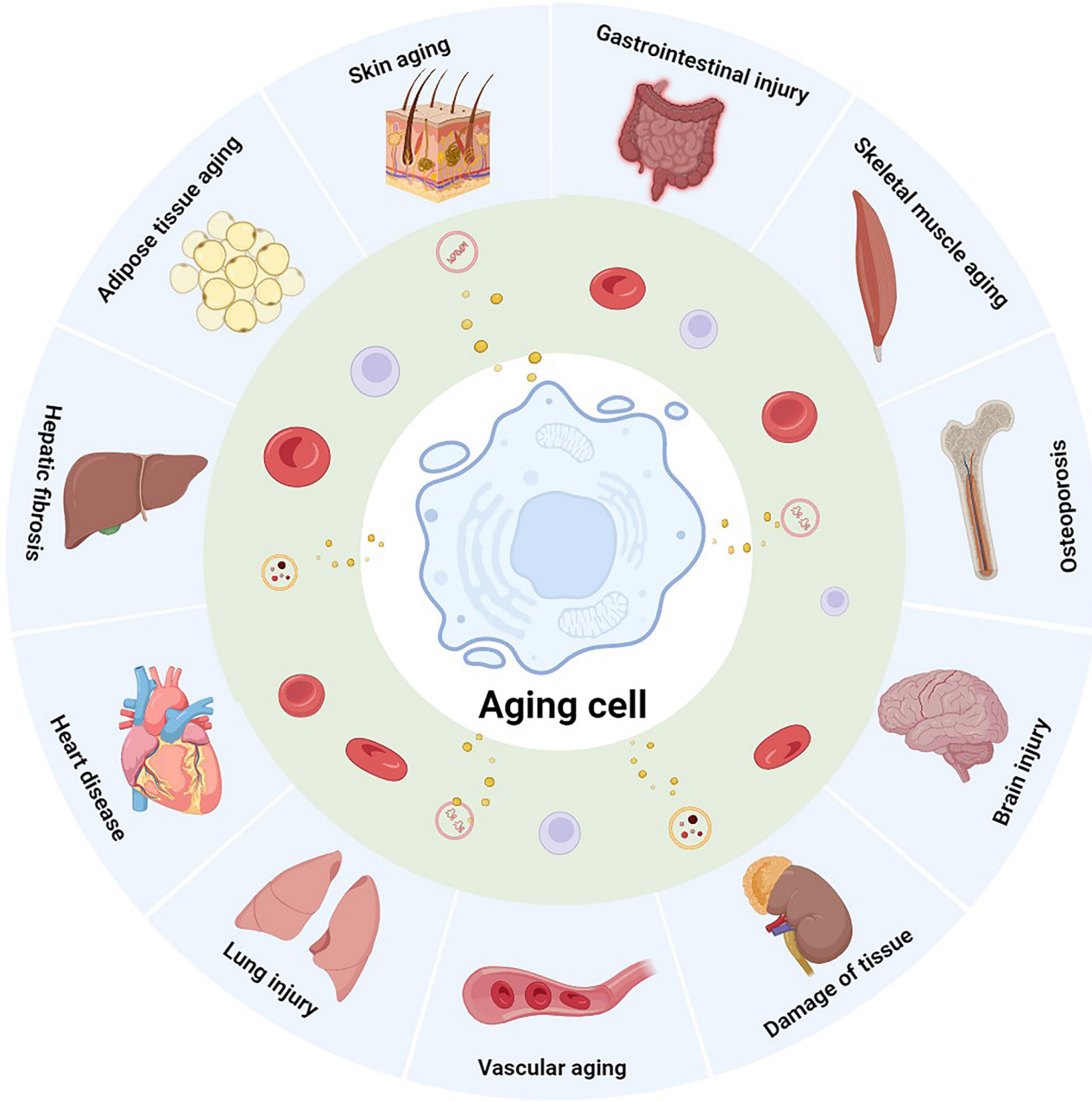

Figure 3.
These EVs can travel beyond the local bone environment through the circulatory system, affecting distant cells, tissues and organs.

taken up by neighboring cells, they activate inflammatory signaling pathways, including the NF-κB pathway, in recipient cells [48]. This activation enhances osteoclastogenesis—the formation of bone-resorbing osteoclasts—and inhibits osteoblast function, disrupting the balance of bone remodeling. The sustained inflammatory state accelerates bone degeneration, reduces bone mass, and weakens bone structural integrity.

7. POTENTIAL OF EVS IN ORGANELLE REPAIR AND BONE AGING INTERVENTION

7.1. EVs as Therapeutic Carriers

EVs have emerged as promising therapeutic carriers for targeted drug delivery and therapeutic applications (Figure 4). Engineered EVs can be designed to carry specific molecules that target and repair dysfunctional organelles such as mitochondria and the ER in bone cells. By loading EVs with mitochondrial protective agents, antioxidants, or proteins that enhance mitochondrial biogenesis and function, it is possible to restore mitochondrial activity in osteoblasts and osteocytes [49]. Similarly, EVs can deliver chaperone proteins, ER stress modulators, or molecules that enhance protein folding capacity to alleviate ER stress and improve ER function [50]. For instance, mesenchymal stem cell-derived EVs have been shown to carry mitochondrial components and antioxidants that protect recipient cells from oxidative stress [49]. These EVs can enhance mitochondrial respiration, reduce ROS levels, and promote cell survival. By restoring organelle function, EV-based therapies can improve the proliferation and differentiation of bone-forming cells, thereby enhancing bone regeneration and combating age-related bone loss.

EVs possess inherent advantages as delivery vehicles, including biocompatibility, low immunogenicity, and the ability to cross biological barriers. Utilizing EVs to efficiently deliver specific genes or drugs to bone tissue offers a targeted approach to repair organelle functions [51]. Genes encoding for key proteins involved in mitochondrial function, ER stress response, or autophagy can be packaged into EVs and delivered to deficient cells to restore normal function. Autophagy inducers stimulate lysosome activity, enabling the efficient degradation of damaged organelles and misfolded proteins. By enhancing the autophagic process, these inducers promote the clearance of cellular debris, thereby supporting mitochondrial function and helping restore the integrity of cellular structures. Moreover, EVs can be loaded with small-molecule drugs, peptides, or nucleic acids that modulate organelle activities. For example, EVs engineered to carry siRNA or miRNA mimics can regulate gene expression related to organelle function in recipient bone cells [52]. This targeted delivery system minimizes off-target effects and enhances therapeutic efficacy. The use of EVs for drug delivery in bone tissue holds significant potential for treating osteoporosis and other bone diseases associated with aging.

7.2. Multi-Organelle Coordinated Intervention Strategies

Bone aging is a multifactorial process involving the dysfunction of multiple organelles, including mitochondria, ER, and lysosomes. A coordinated intervention strategy that simultaneously targets these

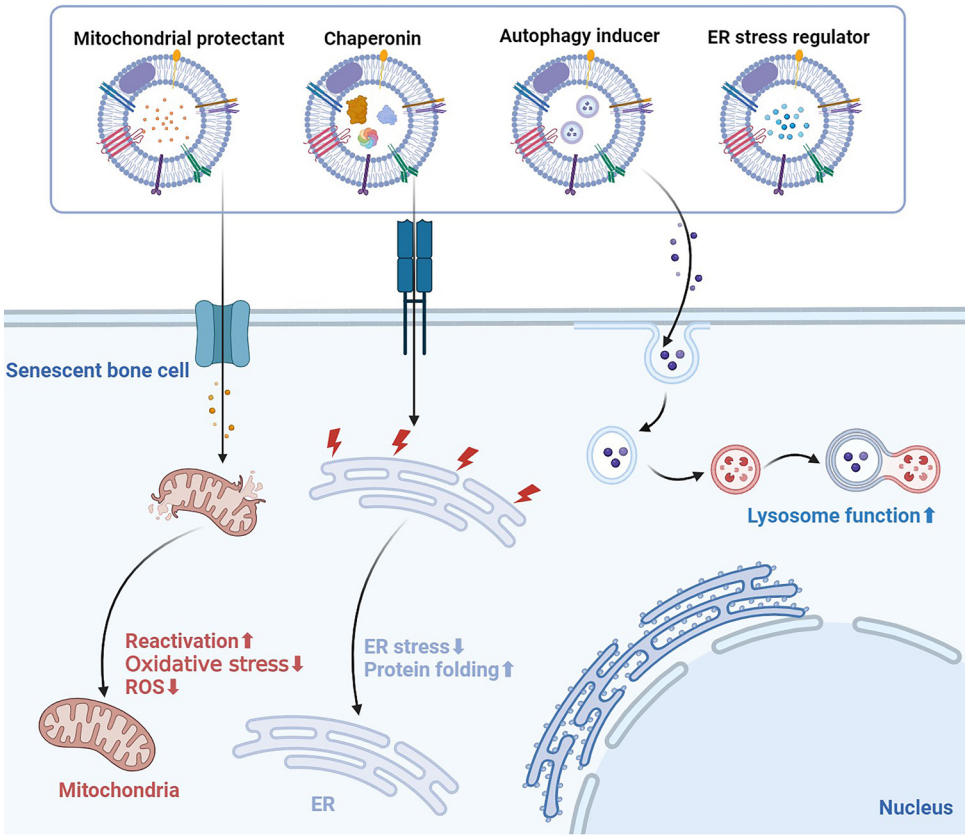

Figure 4.

EVs as a therapeutic carrier. Engineered EVs can deliver therapeutic agents, such as miRNAs, antioxidants, and proteins, to target organelle dysfunction in bone cells. Surface modifications enhance targeting to bone tissue.

organelles may offer a more effective approach to mitigating bone degeneration [53]. Combination therapy involves the use of EVs loaded with a cocktail of therapeutic agents that address the various aspects of organelle dysfunction. For example, EVs can be engineered to carry antioxidants to reduce oxidative stress in mitochondria, chaperone proteins to alleviate ER stress, and autophagy inducers to enhance lysosomal function [54]. By restoring the function of these organelles concurrently, combination therapy can improve cellular homeostasis, promote osteoblast activity, and inhibit osteoclast-mediated bone resorption. Such a holistic approach may lead to significant improvements in bone density and strength in aging populations. Modulating the secretion and content of EVs offers another avenue for influencing communication between bone cells [55]. By altering the signals transmitted via EVs, it is possible to shift the bone remodeling balance toward bone formation and away from resorption. Strategies may include enhancing the production of beneficial EVs from osteoblasts or MSCs while inhibiting the release of EVs that promote osteoclastogenesis. Gene editing tools like CRISPR/Cas9 can be employed to modify donor cells to produce EVs with desired properties [56]. For instance, overexpressing osteogenic miRNAs or proteins in EVs can enhance osteoblast differentiation and activity in recipient cells. Conversely, suppressing the expression of pro-resorptive factors in EVs can reduce osteoclast formation and function. Regulating EV-mediated intercellular communication provides a targeted method to modulate bone remodeling processes and counteract the effects of aging on bone tissue.

8. CONCLUSION

Emerging evidence highlights the crucial role of compromised organelle function, particularly in mitochondria, endoplasmic reticulum, lysosomes, and Golgi apparatus, in propagating degenerative signals through EVs trafficking. Future research should focus on several interconnected priorities, including a comprehensive mapping of organelle-EVs signaling networks within aging bone microenvironments. Research needs to explore how stress-induced changes in organelle structure and function, such as mitochondrial DNA leakage, ER stress response activation, and lysosomal enzyme dysregulation, affect EVs biogenesis and cargo selection. This involves characterizing the temporal progression of organelle-derived damage signals through distinct EVs subpopulations and understanding their differential impact on bone cell homeostasis. Additionally, mechanistic studies should clarify how EV-mediated cross-talk influences the bone remodeling process during aging, particularly the dual role of senescent cell-derived EVs in promoting osteoclast activation and suppressing osteoblast differentiation through paracrine signaling. Advanced single-vesicle analysis techniques can provide insights into how aging alters EVs surface proteomes, affecting target cell specificity, especially in mesenchymal stem cell-osteocyte communication. Furthermore, the therapeutic potential of engineered EVs should be systematically explored, with bioengineering approaches focused on EVs modification for organelle-targeted therapy. These approaches include developing EVs scaffolds carrying mitochondrial-protective miRNAs or ER stress inhibitors, engineering bone-targeting EVs surfaces, and optimizing EVs loading strategies for lysosomal enzyme replacement. Simultaneously, standardized protocols for EVs isolation and characterization must be established to address current challenges related to EVs heterogeneity. Critical obstacles in this field include technical limitations in tracking organelle-specific EVs biogenesis in vivo, the dynamic reciprocity between age-related extracellular matrix changes and EVs biodistribution, and physiological barriers to targeted EVs delivery in hypovascular bone tissue. Additionally, a deeper understanding of EVs cargo sorting mechanisms under oxidative stress conditions is required. Overcoming these challenges will necessitate interdisciplinary collaboration, integrating advanced imaging modalities, multi-omics approaches, and biomimetic 3D bone culture systems. Special attention should be given to the development of spatial transcriptomics platforms that can resolve EV-mediated signaling gradients within aging bone niches. This shift in research focus, from single-organelle analysis to systemic EVs communication networks, promises to transform our understanding of skeletal aging, offering the potential for novel diagnostic biomarkers and precision therapies for osteoporosis and other age-related osteopathies.

Looking forward, there is substantial potential in developing EV-based organelle repair methods and multi-organelle coordinated therapeutic strategies to delay or reverse bone aging. Advances in nanotechnology and bioengineering could enable the design of engineered EVs that specifically target dysfunctional organelles within bone cells. Combination therapies that simultaneously address mitochondrial dysfunction, ER stress, and lysosomal impairment may offer more effective interventions. Moreover, modulating EV-mediated intercellular communication presents a promising avenue for restoring bone homeostasis and enhancing bone regeneration. Research into manipulating the secretion and cargo of EVs could lead to innovative treatments that counteract the detrimental effects of aging on bone tissue.

In summary, this review highlights the critical roles of organelles and EVs in the process of bone aging. By elucidating the mechanisms of organelle dysfunction and EV-mediated signaling, we gain valuable insights into the pathogenesis of bone degeneration. Addressing the current challenges through focused research efforts will pave the way for developing novel therapeutic strategies. Future studies that harness EV-based therapies and multi-organelle approaches hold promise for improving skeletal health and reducing the burden of osteoporosis and fractures in the aging population.

COMPETING INTERESTS

The authors declare that there are no relevant financial or non-financial competing interests in relation to the manuscript submitted for publication. This includes, but is not limited to, employment, consultancies, stock ownership, honoraria, patents or patent applications, and grants or other funding.

ACKNOWLEDGEMENT

The authors acknowledge the financial support from National Natural Science Foundation of China (82230071, 82172098, 82472444), Shanghai Committee of Science and Technology (23141900600, Laboratory Animal Research Project), Shanghai Clinical Research Plan of SHDC2023CRT01, and General Project of Jiangsu Province Natural Science Foundation (BK20231218).

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