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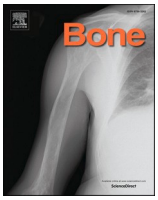

Review Article

Skeleton-derived extracellular vesicles in bone and whole-body aging: From mechanisms to potential applications

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ABSTRACT

The skeleton serves as a supportive and protective organ for the body. As individuals age, their bone tissue undergoes structural, cellular, and molecular changes, including the accumulation of senescent cells. Extracellular vesicles (EVs) play a crucial role in aging through the cellular secretome and have been found to induce or accelerate age-related dysfunction in bones and to contribute further via the circulatory system to the aging of phenotypes of other bodily systems. However, the extent of these effects and their underlying mechanisms remain unclear. Therefore, this paper attempts to give an overview of the current understanding of age-related alteration in EVs derived from bones. The role of EVs in mediating communications among bone-related cells and other body parts is discussed, and the significance of bones in the whole-body aging process is highlighted. Ultimately, it is hoped that gaining a clearer understanding of the relationship between EVs and aging mechanisms may serve as a basis for new treatment strategies for age-related degenerative diseases in the skeleton and other systems.

1. Introduction

The skeleton is the body's largest organ and makes up more than 20 % of an adult's body weight [1]. Bones support the body mechanically, protect the inert organs physically, are metabolically active, and participate closely in systemic mineral homeostasis and hormone regulation. Unfortunately, as an individual ages, impairment of bone mass, quality, and function, which contributes to skeletal dysfunction, becomes evident [2]. Today, age-related bone diseases are a prevalent health issue and contribute to the public financial burden. However, their underlying mechanisms have not yet been fully clarified and there is a lack of availability of strategies for practical therapy.

The skeletal system consists of bone, bone marrow, and other connective tissues. Bones are composed of a bone matrix and various bone cells at different developmental stages. Bone cavities are occupied by bone marrow, a semisolid tissue that contains multiple cell types. These developing or mature cells originate mainly from bone marrow mesenchymal stem cells (BMSCs) and hematopoietic stem cells (HSCs). BMSCs can differentiate into most marrow stromal cell lineages – such as osteoblasts, bone marrow adipocytes, and other chondrogenic and myogenic populations [3]. Bone marrow (BM) is highly vascularized – a large network of blood vessels forms BM's microcirculation, serving as a highway for the delivery of oxygen, nutrients, and biochemical signals [4]. The intricate modulation of lineage commitments and interactions among these cell types via locally produced soluble factors constitutes the bone marrow microenvironment (BMME) [5]. The BMME is a highly heterogeneous and dynamic system that plays a key role in skeletal development, hematopoiesis, immunoregulation, and even systemic homeostasis. The properties of bone matrix are determined by the co-regulation of several cell types and signal factors [6]. Physiological bone remodeling reaches an equilibrium through the deposition of new bone by osteoblasts and resorption of the old, impaired bone by osteoclasts [7]. Osteocytes have long, dense dendritic processes and reside within a network of canaliculi; they act as mechanosensors and play an important role in bone homeostasis.

Cellular senescence is a general cell state associated with aging and

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various kinds of pathological processes that are closely related to age-related diseases [8]. Numerous studies have emphasized the role of senescent bone cells in the acquisition of a senescence-associated secretory phenotype (SASP). Targeting cellular senescence and SASP has been demonstrated to improve or reverse the aging condition of bones [9–11]. Moreover, EVs, which are thought to play a role in SASP [12,13], are becoming an emerging field of interest. EVs are tiny, lipid-bilayer-bound particles that vary in size, origins, and components and have two main subtypes: exosomes and microvesicles (MVs) [14]. A better understanding of the role of senescent EVs in cell-to-cell communications within the bone microenvironment and systematic circulation has the potential to serve as the basis for a novel perspective on aging and bone aging. However, the intrinsic connection between senescent cell-derived EVs and bone aging has not yet been studied extensively.

This review will provide an overview of recent research on the alterations of EVs with age and the functions of senescent EVs in aging bones and organisms. We discuss the probable mechanisms of senescent EVs involved in bone and whole-body aging and highlight findings showing possible ways to alleviate bone aging, which may open up new translational treatment paradigms for age-related bone diseases and other degenerative diseases.

2. Skeletal aging and cellular senescence in bone

With chronological aging, the quality and function of the skeleton suffer dramatic impairments. Aged bones are characterized by irreversible bone loss, as decreased new bone formation and increased old/damaged bone resorption lead to a negative balance [15]. As a common progenitor cell of osteoblasts and bone marrow adipocytes, the decline in BMSC function also influences bone metabolism through their propensity for adipogenic differentiation. This leads to the aberrant expansion of bone marrow adipose tissue (BMAT), which constrains the space for other cells to proliferate. Because BMAT is known to function as an endocrine tissue, the resulting increase during aging in the secretion of a series of proinflammatory cytokines and adipokines generates an inflammatory microenvironment within the bone [16–18] and negatively impacts neighboring osteoblasts, thus accelerating age-related bone loss [19].

Cellular senescence [20,21] is effectively a cell destiny, similar to replication, differentiation, and apoptosis. It is characterized by irreversible proliferative arrest while still maintaining cell viability, as the result of elevated expression of the cell cycle inhibitors CDKN2a or CDKN1a. A series of survival stresses [22], including telomere attrition, DNA damage, and mitochondrial dysfunction, drive a cell into a senescence program. Despite the arrest of their growth, these senescent cells remain metabolically active. They excessively synthesize and secret a complex mixture of bioactive molecules, such as a range of signaling factors (cytokines, chemokines, and growth factors) and tissue-destroying proteases (serine proteases and matrix metalloproteinases (MMPs)), commonly referred to as SASP [23]. The SASP factors were thought to transmit signals of senescence to local and systemic cells and activate the immune system, subsequently resulting in stem cell dysfunction and tissue damage [24].

It is now well established that senescent cells accumulate in various tissues [20,25] over time, leading to in vivo age-related declines. Accordingly, it is becoming clear that the biological significance and pathogenic repercussions of senescent cells will be amplified with advanced age. The transplantation of even a small number of senescent cells can induce physical impairment [26,27]. For example, when 200,000 radiation-induced senescent fibroblasts were transplanted into the knee region of young mice, osteoarthritis (OA)-like phenotype surfaced, with the appearance of leg pain sensitivity, impaired mobility, and radiographic and histological changes [28].

The majority of these mechanisms are also present in bone cells [29,30]. As osteocytes constitute the most prevalent cell type, accounting for about 90 % of the bone matrix [31], a typical histologic change in bone aging is reduced osteocyte abundance. However, a notable finding is the significant rise in the proportion of senescent osteocytes within the cortices of older bones compared to young bones [32]. Several studies [32–34] have confirmed that osteocytes play a predominant role in bone aging. Despite constituting a minor fraction (approximately 11 % in aged mice) of the total osteocyte population, senescent osteocytes become the main source of SASP within the BMME. Nevertheless, Khosla's lab [35] has further identified and subdivided senescent cells in aged bone tissues at the single-cell level. They found that osteolineage cells seem to be the main source of SASP factors in the osteogenic niche, whereas monocytic cells seem to be the main source in the hematopoietic niche.

The negative effects of SASP factors on bone homeostasis have been clarified. For example, an in vitro experiment [36] showed that MLO-Y4 cells, a type of osteocyte-like cells, lacked mechanical responsiveness when co-cultured with conditioned media from senescent osteocytes, where IL-6 played a key role through P2X7 inhibition. The pathological progress of osteoarthritis has also been found to be related to the negative effects of SASP factors on bone homeostasis. The senescent mesenchymal stromal cells in human osteoarthritic cartilage (OA-MSCs) have been found to generate interleukin (IL)-1β, IL-6 and IL-8 and CXC motif chemokine ligand (CXCL) 1, 5 and 6, passing on a chronic inflammatory state to chondrocytes and deteriorating the cartilage matrix. As research progresses, in addition to classical protein factors, it becomes clear that EVs are also a product of the secretion phenotypes of aging bones. Given the variability of the structures and cargoes involved, it is evident that a better understanding of EVs will contribute to the elucidation of aging mechanisms, as well as to the development of cell-free therapeutic strategies.

3. Senescent EVs in the context of bone aging

Since Valadi et al. [37] first demonstrated that EVs enable the transfer of functional RNA to target cells, they have been recognized as crucial mediators in cell-to-cell communication, particularly over long distances. Because senescent cells and those accumulated in aged tissue undergo tremendous changes in phenotype and function, EVs derived from them always have distinguishing features and effects. We defined these as senescent EVs in this article. Cellular senescence is widely recognized as a potent mechanism in tumor suppression. However, SASP including extracellular vesicles has been found to promote tumorigenesis [38,39], which provides senescence with a Janus-faced role in cancer. Furthermore, it was through proteomic analysis of proteins in senescent EVs that some unknown SASP factors were discovered [40]. Thus, in-depth research on EVs is becoming a hot spot in research aimed at enriching our understanding of the potential mechanisms of cellular senescence and aging.

3.1. Changes in EVs with bone aging

As a crucial element of SASP, EVs generated from senescent bone cells manage to mirror the pathological state of their origins from the standpoint of bone secretion; thus, it appears that some features of these EVs undergo subtle changes (Table 1).

Since Lehmann et al. [41] first reported a senescence-associated increase in EV secretion, this phenomenon seems to be a common feature of cellular senescence. Enhanced EV secretion, which is more than 10-fold higher, has been observed in several senescent cell types (e.g. cancer cells, fibroblasts, and epithelial cells) and in vitro senescence models driven by serial passaging, oncogenic RAS activation, and radiation [39,41,42]. Jeon et al. [43] isolated chondrocytes from OA patients and enriched senescent populations by flow cytometry sorting. They found that the number of small EVs released from cultures with high concentration (65 %) of senescent chondrocytes is increased by more than 5 times compared to those from normal cultures. This increase in EV secretion has also been demonstrated with natural,

Table 1
Published Studies on Age-related alterations of senescent EVs derived from aged bone cells.

Cell/ Tissue	Organisms	Age	Methods: isolated and identified	EV characteristics	Findings	Reference
BMSCs	Rats	young (14 days old) vs. old (270 days old)	·Ultracentrifugation: 2000 ×g for 10 min at 4 °C, filtration through 0.22 μm filter, 100,000 ×g for 2 h at 4 °C ·TEM, NTA	Concentration Size Cargos (proteins)	↑EV concentration with age. No difference in EV size with age. ↓the protein to particle ratio with age.	[44]
BMSCs	Rats	newborn (0 days old), infant (7 days old), young (14 days old), pre-pubertal (35–38 days old), pubertal (45 days old) and adult (108 days old)	·Ultracentrifugation: 1500 rpm for 10 min at 4 °C, filtration through 0.22 μm filter, 100,000 g for 2 h at 4 °C ·TEM, NTA	Concentration Size Cargos (proteins & miRNAs)	↑EV concentration with age. No difference in EV size with age. ↓the protein to particle ratio with age. miR-146a, miR-155 and miR-132 decreased with age; miR-335 highest in the adult group and miR-21 highest in the pre-pubertal group.	[53]
BMSCs	Human	young (median age: 22 years) vs. aged (median age: 69 years)	·exoEasy Maxi Kit ·TEM, NTA, Western blot (CD63, CD81, Flotillin-1, Calnexin)	Concentration Size Cargos (miRNAs)	No difference in EV concentration and size with age. ↓the total amount of miRNA with age. miR-29a and miR-34a increased with age.	[45]
Whole bone marrow	Mice	Young (6–8 weeks old) vs. old (24–26 months old)	·Ultracentrifugation Ultracentrifugation:2000 ×g for 30 min, 100,000 ×g for 1 h · NTA	Concentration Size Cargos (miRNAs)	↑EV concentration with age. No difference in EV size with age. miR-29a, miR-24, and miR-21 increased and miR-105 decreased with age.	[49]
OLCS	Mice	Young (3 months old) vs. old (20 months old) female	·Ultracentrifugation:300 ×g for 10 min; 2000 ×g for 30 min; 10,000 ×g for 30 min; 150,000 ×g, 2 h at 4 °C ·TEM, LSM, Western blot (CD9, CD81)	Cargos (proteins)	Classical SASP proteins increased and functional proteins that regulated bone homeostasis decreased with age.	[31]

chronological aging. For example, an in vitro assay [44] reported that production of BMSC-derived EVs from rats showed an age-dependent increase (52 %); To mention it, above research data is based on traditional differential ultracentrifugation (UC) and Nanoparticle tracking analysis (NTA) to isolate and characterize EVs. Several experiments [45,46] showed there was no significant difference in EV concentration of BMSCs between the young group and aged group when some isolation kits (e.g. exoRNeasy Maxi Kits, Total Exosome Isolation kits) were applied. As reported by Tian et al. [47], compared to UC, EV preparations by kits showed much higher (two to four orders of magnitude) particle concentrations and lower purity of EVs, in which non-vesicular contaminants such as lipoprotein cannot be differentiated by particle ensemble-averaged approaches. Therefore, different separation techniques with varied isolation efficiency might disturb the characterization of EVs.

Another consideration is complex physiological and pathological regulations within the internal environment when it comes to in vivo concentration of EVs [48]. At the tissue level, the number of whole bone marrow (WBM) derived EVs from old donors had an approximately 2-fold increase compared to young WBM [49]. However, Eitan et al. [50] launched a cross-sectional and longitudinal study and found concentration of EVs circulating in the blood declined with age, which is partly ascribed to increased internalization of EVs by B cells compared with EVs from younger individuals.

The cargo of EVs also changes with age. The overall particle ratios of senescent EVs seem to decrease with increasing donor age; the concentration of protein per particle of BMSC-EVs from older rats was lower than that of the younger group, which was determined by NTA assay [44]. Likewise, less total amount of miRNA was also observed in BMSC-EVs from older people [45]. But in terms of specific cargoes, variation in their content could reflect age-associated pathologies in most cases demonstrated. Osteocytes, the most numerous cell populations in bone, have long, dense dendritic processes that reside in a network of canaliculi and form a cellular communication network termed the osteocytic lacunar canalicular system (OLCS) [51,52]. As such, upregulation of several classical SASP proteins, including transforming growth factor (TGF)-β2, osteoclastogenesis inhibitory factor (OPG), and MMP9, has been shown in exosomes from aged OLCS, which potentially accelerates bystander senescence and mediates bone metabolism. Conversely, key molecules that mediate the osteogenic process, as well as members of the BMP and Wnt signaling pathways are inhibitory, which is consistent with the gradual loss of dominance of bone cells with aging [31]. The EV-miRNAs profile also shows age-dependent differences. To investigate the age-dependent immune responsiveness of BMSCs, researchers [53] isolated and cultured BMSCs respectively from four age groups of rats and collected their secreted EVs. They found that EVs from the prepubertal group displayed the most significant expression of miR-21-5p. Silencing this miRNA resulted in reduced expression of Toll-like receptor 4 (TLR4), which activated downstream Wnt3a and Wnt5a signaling pathways to regulate BMSC proliferation and osteogenic differentiation. In contrast, exosomal miR-335 was expressed at the highest level in the adult group, which in turn suggested cellular senescence and a loss of proliferative capacity.

It should be noted that cellular senescence is not the only cause for age-dependent changes in EVs during bone aging. Apoptosis is a kind of cell fate that is defined as a programmed cell death, which normally permits self-renewal of bone cells and maintains bone balance. The apoptotic cell also could release kinds of EVs, widely known as apoptosis-derived EVs (ApoEVs). At the tissue level, bone aging is a gradual process in which the states of cell populations are at different stages timely and spatially [54]. It is proved that the prevalence of osteoblast and osteocyte apoptosis increases progressively with age in mice [55]. The increase of osteocyte apoptosis is also observed in several pathological conditions, such as disuse atrophy [56], sex hormone deficiency [57], and inflammation [58]. Therefore, the pool of senescent EVs derived from aged bones might contain the apoptotic EVs. At the cell level, senescence and apoptosis are concurrently involved in specific processes or stress reactions [59], which is related to the level of survival stress, the modulation of the p53-p21 axis, and transduction of PTEN-AKT signaling [60]. Therefore, when figuring out the characteristics and function of EVs in a senescence model, the state of the cell population and the heterogenicity of senescent EVs should be taken into account.

3.2. Roles of senescent EVs in the context of bone aging

As mentioned above, alterations in certain properties of the senescent EVs contribute to age-related deterioration of physical function. Researchers have observed these changes and their corresponding biological effects in EVs derived from various kinds of senescent cells, both in bone tissue and in other body parts (Table 2 and Fig. 1).

3.2.1. Within the bone cell population

In vitro evidence has proved that EVs derived from aged bone tissues or cells carry harmful contents and have the potential to generate negative impacts of bone aging. Davis et al. [46] observed that BMSCs exhibited decreased osteogenic differentiation capacity and increased oxidative stress upon endocytosis of EVs obtained from the bone marrow interstitial fluid of aged rats. Likewise, Fafián et al. [44] reported that when incubated with aged BMSC-derived EVs, the expression of *Nanog* and *Oct4*, reflecting pluripotency, of young BMSCs was suppressed, while the degree of cellular senescence was raised. Further investigation demonstrated that the knockdown of miR-188-3p in young BMSC-derived EVs induced rejuvenation of aged BMSCs, through an increase in RICTOR levels. Therefore, miR-188-3p could be a novel target for the treatment of osteoporosis (OP) in the elderly.

The interplay between BMSCs and osteoclasts has been experimentally shown to depend on transferring EVs in a paracrine manner. As reported by Xu et al. [61], miR-31a-5p, which has an increasing expression in aged BMSCs, functioned to influence lineage commitment towards adipocytes through special AT-rich sequence-binding protein 2 (SATB2). Moreover, miR-31a-5p-enriched EVs released by aged BMSCs can promote the differentiation and activity of osteoclasts by stimulating the RhoA pathway. Li et al. [62] designed a Transwell coculture system to prove that the internalization of osteoclast-derived exosomal miR-214-3p leads to the inactivity of osteoblasts and in vivo injection of osteoclast-derived exosomes verified its bone-target specificity. Further targeted inhibition of osteoclastic miR-214-3p survived the bone formation of aging OVX mice. Interestingly, some researchers [63] have observed that miR-214-3p also has a stably high expression in salivary exosomes only in old donors. More importantly, the level of miR-214-3p is positively correlated with several clinical indicators of periodontal diseases, including probing pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP). Considering the dominance of osteoclasts in the progression of periodontitis, it is indicated that both age-related bone diseases may share mechanistic links. Accordingly, EVs, with their miRNA cargoes, can act as messengers to transfer the senescent signals among bone cells, weaving an intricate network of bone aging.

3.2.2. Bone and vasculature

Bone is not only a highly calcified tissue with an abundant matrix but is also richly vascularized. It is widely acknowledged that there is a connection between bone formation processes and the angiogenesis processes [64–67]. For instance, the pro-aging miR-188-3p found in BMSC-EVs mentioned above also exerts a negative effect on the formation of type H vessels, which causes a reduction in bone mass and a delay in bone regeneration [68]. Li et al. developed an in vitro model of senescent osteoblast induced by D-galactose. Senescent osteoblasts have been shown to release exosomes carrying overexpression of miR-139-5p. Increasing miR-139-5p decreases vascular endothelial cell growth and migration, and further ruins vasculature formation by knocking down its target T-box 1 (TBX1) [69]. In contrast, another in vitro experiment, reported by Weilner, revealed that senescent endothelial cells secreted CD63-positive MVs to inhibit the osteogenic process of adipose tissue-derived MSCs (ASCs), in which microvesicular miR-31 was found to play a pivotal role [70]. Furthermore, miR-31 enriched in plasma MVs from elderly donors also showed the osteogenic inhibitory effect, suggesting its contribution to impaired osteogenesis during aging in vivo. However, the supplementation of miR-31 has been found to promote the survival and proliferation of BMSCs exposed to lipopolysaccharide (LPS) [71], although influences on osteogenesis ability have not been mentioned. Therefore, more research is needed to clarify its function in different types of mesenchymal stem cells. This reciprocal signaling in the coupling of osteogenesis and angiogenesis via membrane vesicles, nevertheless, calls for the investigation of the synchronicity of skeletal diseases and vascular diseases during natural aging.

Recently, Xie et al. [72] revealed a novel 'bone-vascular axis' in which aged bone matrix-derived EVs induced fat accumulation in the bone marrow lumen and vascular calcification by transporting miR-483-

Table 2
Published Studies on Senescent EVs-mediated crosstalk within the bone tissues and between the skeleton and other organ/systems.

Sources	Kinds	Specific cargos	Targeted genes/ mechanisms	Recipient cells	Functions	Reference
Originated from aged bones	BMSCs	miR-344a, miR-133b-3p, miR-29↓	E-cadherin↓ α-SMA↑	HK2 cells	TGF-β1-mediated EMT Renal fibrosis	[92]
	BMSCs	miR-29b-3p	SIRT1	3 T3-L1 adipocytes, C2C12 myocytes, Hepatocytes	Insulin Resistance	[86]
	BMSCs	miR-31a-5p	SATB2	Osteoclasts	Osteoclastogenesis	[61]
	BMSCs	–	Akt/mTOR	Young BMSCs	Cellular senescence	[44]
	Osteoclasts	miR-214-3p	ATF4	Osteoblasts	Suppressed osteogenesis	[62]
	Osteoblasts	miR-139-5p	TBX1	Endothelial Cell	Affected angiogenesis	[69]
	Osteoarthritic chondrocytes	miR-449a-5p	ATG4B	Macrophages	Autophagy inhibition and Mature IL-1β production	[82]
	Osteoarthritic chondrocytes	Cx43	NF-κB, ERK1/2	Chondrocytes, bone cells and synovial cells	Increased cellular plasticity and cellular senescence	[77]
	Chondrocytes	miR-27b, miR-199a, miR-185, miR-23b	–	–	Cartilage homeostasis dysregulation	[43]
	Bone matrix	miR-483-5p	PPARγ	BMSCs	Adipogenesis	[72]
		miR-2861	RUNX2	VSMCs	Mineralization	
	Bone marrow interstitial fluid	miR-183-5p	Hmox1	BMSCs	Suppressed osteogenic differentiation	[46]
Originated from other aged organs	Endothelial cells	miR-31	Frizzled-3	BMSCs	Inhibited osteogenic differentiation	[70]
	Serum	C24:1 ceramide	–	BMSCs	Senescence	[93]
	Plasma	Vesicular Galectin-3↓	RUNX2	MSCs	Reduced osteo-inductive potential	[96]
	Muscle	miR-34a	SIRT1	BMSCs	Senescence	[87]
	AD Brain	miR-483-5p	Igf2↓	BMSCs	Adipogenesis	[91]

AD, Alzheimer's disease.

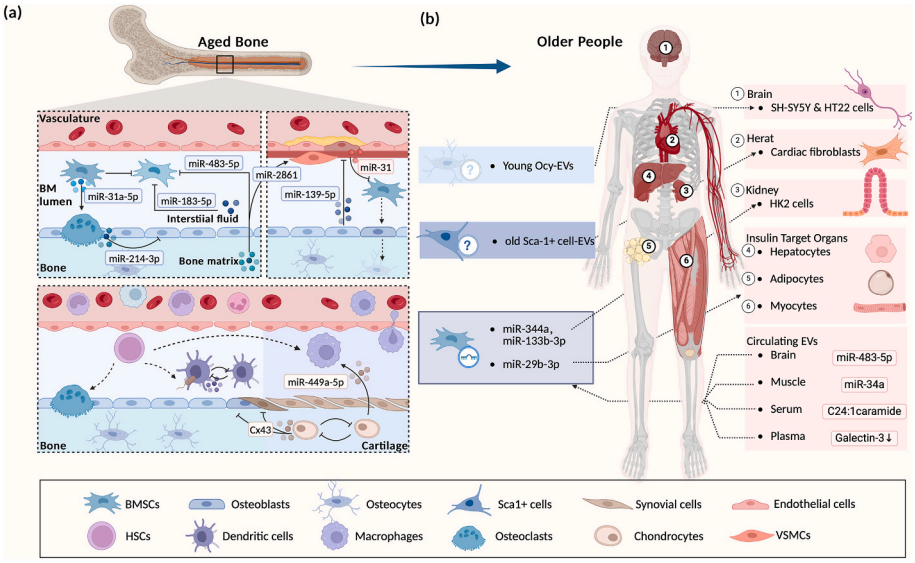

Fig. 1. Senescent extracellular vesicles (EVs)-mediated intercellular or inter-organ communication within aged bone tissue (a) and between the skeleton and other organs/systems (b). (a) In an aged bone microenvironment, crosstalk of senescent EVs among bone/cartilage resident cells triggers cascades of cellular senescence. The delivery and exchange of their functional cargoes facilitate anti-osteogenic activities, bone marrow microcirculation dysfunction, and immunosenescence. (b) In the aging human body, EVs derived from bone tissue exert a long-distance influence on various tissues/organs, whereas EVs derived from kinds of tissues/organs also pass through the circulatory system into the skeleton and affect bone homeostasis.
(Created with BioRender.com.)

5p and miR-2861 into BMSCs and vascular smooth muscle cells (VSMCs), respectively. VSMCs, the major constituents of vessel walls, are known for their pivotal role in the development of atherosclerosis, restenosis, and pulmonary hypertension [73,74]. Consequently, research in this area provides a persuasive explanation for the phenomenon of reduced bone density accompanied by increased arterial stiffness in the elderly [73] and can offer a new perspective on a common therapeutic target for OP and cardiovascular diseases.

3.2.3. *Cartilage/bone and immune system*

The interplay between bone and the immune system is a developing field. Bone tissue is considered to constitute the osteoimmune system, for which HSC niches in the BM are the primary birthplaces of all immune cells [75]. In the shared microenvironment, bone homeostasis is easily influenced by the prolonged or abnormal immune response that can come with aging. OA is a common age-related osteoimmune disorder characterized by degenerative changes in the whole joint, including articular cartilage, subchondral bone, and synovium [76]. In OA progression, senescent chondrocytes are involved in cartilage extracellular matrix (ECM) degradation and persistent inflammation. Eirín et al. [77] identified overexpression of transmembrane protein connexin 43 (Cx43) in EVs secreted by osteoarthritic chondrocytes. In vitro coculture demonstrated that exosomal Cx43 could induce senescence in chondrocytes, bone cells, and synovial cells, but the in vivo effect is to be proven by further experiments. Jeon's group [43] revealed that the removal of SnCs from old mice reduced the secretion of EVs from chondrocytes and altered multiple miRNAs (e.g., miR-27b, -199a, -185 and -23b) in synovial fluids. These miRNAs are related to cartilage homeostasis dysregulation. Apart from degeneration and breakdown of joint tissues, EVs also affect the immunoregulatory function of the joint microenvironment. Synovium is a specialized membrane, consisting of intra-articular vessels, such as blood vessels, lymphatic vessels, and nerves. Synovitis has been demonstrated to be an independent factor in the progression of OA [78,79], in which the synovial macrophages play a crucial role [80,81]. A recent study [82] has found that osteoarthritic chondrocytes can liberate exosome-like vesicles packaging of miR-449-5p into synovium and then activate macrophages to produce mature IL-1β. Intra-articular injection of the vesicles further aggravates cartilage destruction and synovial inflammation in OA mice.

Alveolar bone loss is another inflammatory lesion that is common in advanced old age and is mainly caused by disequilibrium between oral microbial invasion and immune cell deregulation in the periodontium. *Porphyromonas gingivalis* (*P. gingivalis*) is a keystone pathogen and a significant contributor to periodontitis. Dendritic cells (DCs) are thought to be powerful APCs that serve as the first line of defense against infection in the oral mucosa [83]. Elsayed et al. [84,85] found that when exposed to *P. gingivalis*, bone marrow-derived DCs (BMDCs) underwent premature senescence and function impairment, in which BMDCs from old mice became much less responsive to inflammatory stimuli. Additionally, these infected BMDCs also exerted a bystander effect on surrounding cells to amplify the immune senescence by enhancing the secretion of exosomal SASP. This suggests that immune silence under infectious conditions may promote the progression of periodontitis and that an EV-mediated signaling pathway plays a potential role.

3.2.4. *Bone and distal organs via circulation*

As it is closely involved in hormone metabolism and systemic regulation as an 'endocrine organ', the skeleton sends EVs into circulation and, of course, exerts a biological effect on distal organisms. Through paracrine activity, exosomal miR-29b-3p generated by BMSCs from aged mice was found to impair cellular insulin sensitivity in myocytes, adipocytes, and hepatocytes, conservatively binding to the 3′ UTR of *SIRT1* mRNA [86]. Interestingly, overexpressing miR-34a, which is found in skeletal muscle and in circulating EVs generated from muscle, induced senescence in BMSCs, with a reduction in SIRT1 at both the mRNA and protein levels [87], although the consequent impact on the stem cell population in bone has not been investigated. SIRT1, a key modulator in numerous metabolic pathways, not only has a positive effect on glucostasis [88] but also facilitates ossification through upregulated osteogenic differentiation factors such as RUNX2 [89] and downregulated oxidative stress [90]. Meanwhile, bone-muscle interaction is an emerging field in geriatrics, as bone fragility always coexists with muscle atrophy during aging. Therefore, the research mentioned above reveals possible mechanisms of synergistic senescence in the musculoskeletal system and provides insight into OP and sarcopenia, both of which are common age-related diseases.

Likewise, the brain-bone axis has also attracted attention, as the central nervous system is actively involved in bone remodeling. A research group [91] revealed the bone-targeting ability of brain-derived EVs (B-EVs). These B-EVs from adult AD mice (AD-B-EVs) modulated the cell fate of BMSCs and exhibited anti-osteogenic and pro-adipogenic effects. Then, miR-483-5p was confirmed as a major type of functional cargo in AD-B-EVs that downregulates skeletal metabolism. This specific miRNA was also enriched in EVs derived from an aged bone matrix and exerted similar effects [72], indicating a possible therapeutic target for AD patients with OP.

In addition, BMSCs have been shown in several investigations to contribute to renal tissue healing. Moreover, various studies have attempted to apply exosomes from naturally healthy or engineered BMSCs to renal fibrosis. However, it was found that [92] the inhibitory effects of MVs from elderly rats on TGF-induced EMTs were much less pronounced than those of MVs from younger rats. The follow-up microarray analysis of microvesicular miRNAs displayed several downregulated components, including miR-344a, miR-133b-3p, and miR-294, indicating their role in inhibiting TGF-β1-mediated HK2 cell EMT.

When a pool of EVs is released and wanders into systematic circulation, these circulating EVs also deliver biological messages when communicating with bone tissues. This is exemplified in the work undertaken by Khayrullin and his group [93], in which EVs purified from a serum sample of older women showed a notable increase in C24:1 ceramide, which enabled the acceleration of BMSCs senescence in vitro. C16:0 ceramide, another member of the very long-chain ceramide family, was previously found to enrich osteoblasts and suppress bone formation [94]. A recent study [95] also showed that the age-associated accumulation of C18:0 and C24:1 ceramides in plasma and BM directly stimulated osteoclastogenesis and bone resorption. In contrast, the reduction of Galectin-3 in elderly plasma-derived EVs lowered the osteogenic potential of MSCs, mainly due to limited activation of the Wnt/β-Catenin pathway [96].

4. The potential value of senescent EVs in clinical applications

Nowadays, EVs are thought to be promising candidates in the diagnosis, prediction, and treatment of diseases because of their capacity to circulate through bodily fluids and carry critical bioactive molecules. Therefore, altered cargoes in senescent EVs can be either biomarkers or therapy targets for aging and aging disorders. On the other hand, considering aging impairments, EVs isolated from a young, healthy environment appear to benefit organisms. For instance, Yoshida et al. [97] have shown that young mouse plasma-derived EVs can reduce senescence and extend health span. The effector, extracellular nicotinamide phosphoribosyltransferase (eNAMPT) is localized exclusively in EVs. Therefore, young donor-associated therapy, a promising candidate for use in achieving anti-aging goals, has become a prosperous research area.

4.1. Age-related bone diseases

A gradual decline in bone mass and a deterioration of bone architecture occurs with age, resulting in many bone-related diseases such as OP, OA, and periodontitis. Based on the above investigation into the EV-mediated interplay among aged bone resident cells, it was determined that most functional vesicular miRNAs (e.g., miR-139-5p and miR-188-3p, which originate from BMSCs, and miR-214-3, which originates from osteoclasts) negatively regulate the process of osteogenesis. Hence, it is possible to use the levels of particular miRNAs to suggest the senescent conditions of bone tissue, and these miRNAs can be employed as knockdown targets to protect against bone aging.

The relative expression of specific miRNAs can also be a feasible indicator for assessing the efficacy of anti-aging therapies. Removing principal senescent cells is one of the main methods for targeting cellular senescence [98]. In the OA mouse models, clearance of SnCs in the younger group significantly decreased the expression of miR-34a in synovial EVs, which was associated with OA progression [43]. However, no apparent differences were detected in the elderly group. The results indicated poor responsiveness to drugs that target SnCs during aging and signified an unfavorable prognosis for OA (Fig. 2a).

BMSC-EVs have been a rising star in acellular therapy because of their advanced functions in immunity modulation, anti-inflammation, and mitochondrial homeostasis. Their altered cargoes and function during aging, which also happens in other types of MSCs, become a concerning problem. ASCs are also powerful candidates for the mass production of EVs. It has been shown that EVs from old ASCs failed to exhibit protective effects in the manner of young ones [99]. Certainly, under in vitro circumstances, the cargoes and biological efficacy of MSC-derived EVs mostly depend on the donors, tissue sources, and culture conditions of the MSCs [34,100,101]. Research [34] utilizing human-induced pluripotent stem cell-derived MSCs (iMSCs) demonstrated that EVs from iMSCs gradually begin to lack potency in immunomodulation as the passage number increases due to altered contents of exosomal miRNAs and proteins. Such an in vitro replicative senescent cell model was also identified in human umbilical cord MSCs, in which those in late passage inhibited pro-osteogenesis function [102]. As for this, insight into aging and cellular senescence could help to set standards for selecting cell donors and engineered targets and to ensure that BMSC-EVs have an optimum effect.

4.2. Cardiovascular diseases

Cardiovascular diseases dominate health issues for very old populations. Age-related phenotypes, including cardiac hypertrophy, vascular stiffness, and fibrosis, are closely related to pathological changes in different types of cardiac cells [103]. Cardiac fibroblasts, one of the principal non-myocyte cell types in the heart, are responsible for ECM remodeling and regeneration, which form cardiac fibrosis [104,105] following ischemic injuries. Previous studies have shown that transplantation of young bone marrow cells (BMCs) reconstituted aged bone marrow and that their homing to the myocardium rejuvenated the aged heart [106]. These BMCs, labeled with stem cell antigen-1 (Sca-1), have a stronger homing efficiency and paracrine ability compared to whole BMSCs [107]. Yeganeh et al. [108] found that the young Sca-1^{+} cells were able to repair the cardiac fibroblast function through an autophagy-mediated paracrine pathway, but that the aged ones did not. This age-dependent difference was later eliminated when autophagy was inhibited in young populations, partly because their secreted EVs lost their beneficial effects on fibroblasts. This better understanding of the far-distance interaction between BM and the heart can provide insight into the underlying mechanisms and promote stem cell therapy improvement.

Similarly, vascular calcification is a common sign in older people, which, contradictorily, often occurs with OP. It has been discovered [72] that EVs collected from the aged bone matrix (AB-EVs) have a dual effect: AB-EVs-treated mice showed a reduction in osteoblast number but an increase in marrow adipocytes in the femur, as well as an increase in calcium deposition in the abdominal aortas. However, alendronate, an inhibitor of osteoclast-mediated bone resorption, was found to restrain the release of AB-EVs in vitro, and later, in aged OVX mice, it rebuilt bone-fat homeostasis and protected against vitamin D3-induced vascular calcification. It has been suggested that alendronate exhibits a new paracrine pathway for deciphering this ‘calcification paradox’ and could lead to a breakthrough in treating comorbid conditions, though more in-depth studies on its in vivo effects on AB-EV release with its specificity are needed (Fig. 2b).

4.3. Metabolic diseases

Type 2 diabetes (T2D) is a typical age-related metabolic disease. The

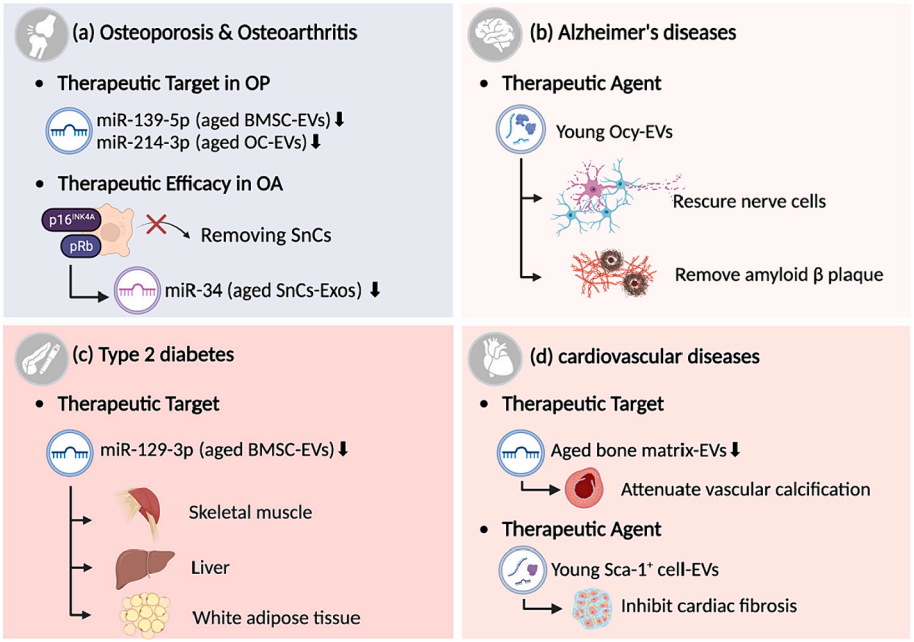

Fig. 2. Potential application values of senescent extracellular vesicles (EVs) derived from bone-related cells/tissues in age-related diseases. It has been clarified that some biomolecules evolved in senescent EVs partake in specific pathological processes, meaning that there is a chance they could become potential therapeutic targets; some of their expression levels are age-dependent and, to some extent, reflect the progression of diseases, so it may be possible to construct clinical testing indexes for them to measure therapeutic efficacy. Young donor therapy is rising because of insights into aging mechanisms, so EVs from young, healthy bodies can be used as therapeutic agents to cure these age-related diseases: (a) Osteoporosis (OP) and Osteoarthritis (OA), (b) Alzheimer's disease (AD), (c) Type 2 diabetes (T2D), and (d) cardiovascular diseases.
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homeostasis of systematic metabolism is gradually disturbed during aging [109], whereby glucose homeostasis, which is of great importance to the body's energy supply, also loses its balance. Numerous and increasing evidence has shown a high prevalence of bone fractures (e.g. hip fractures) in T2D patients with normal to high bone mineral densities [110]. In contrast, aged bone also had detrimental effects on insulin sensitivity, as a recent study reported [86]. Myocytes, adipocytes, and hepatocytes – major cell types that actively participate in the glucose metabolism process – were shown to become resistant to insulin when taking up aged BMSC-EVs. Later, the study found that exosomal miR-29b-3p principally regulated age-related insulin resistance. Thus, intriguingly, the specific inhibition of functioned miRNAs involved in EVs reversed this effect, which will become a therapeutic target in age-related insulin resistance mediation (Fig. 2c).

4.4. *Neurodegenerative disorders*

Neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS), are of particular concern in elderly people. As a common form of age-related dementia, AD is neuropathologically characterized by the formation of β-amyloid (Aβ) plaques and neurofibrillary tangles in the central nervous system during aging [111]. There is currently no treatment for AD. Despite their different phenotypes, AD and OP appear to have strong clinical correlations. Most studies have revealed that OP often precedes cognitive impairment [112]; reciprocally, elderly AD patients always present lower bone mineral density (BMD) and a higher risk of hip fracture [113,114]. Jiang et al. [115] found that EVs isolated from young osteocytes can when introduced through intramedullary injection, travel from bone tissue to brain tissue with AD impairment. Further experiments demonstrated that these EVs can accelerate Aβ cleavage and rescue nerve cells and, therefore, improve cognition in mice and slow down the pathogenesis of AD (Fig. 2d). However, aged osteocytes lose the ability to produce these beneficial EVs. Therefore, these studies strengthened the physiological and pathological relationship between the skeleton and brain and demonstrated the presence of EV-mediated communication in the bone-brain axis.

5. Conclusions and perspectives

Functional cargo in EVs is a focus of research. We have figured out specific cargo including proteins and miRNAs carried by senescent bone EVs in this article. It is of importance to realize that senescent EVs and their functional cargos do not work in isolation. Recent pieces of evidence have underscored this point. Aged bone matrix-derived EVs could be a carrier of two functional miRNAs, miR-483-5p and miR-2861, respectively leading to contradictory effects of anti-osteogenesis in bone tissue and heterotopic ossification in blood vessels [72]. MiR-483-5p is also a key player enriched in AD brain-derived EVs, contributing to the osteogenic inhibition of BMSCs [91]. This situation might explain the mystery of how aging signals spread between bone and other systems. Compared to conventional biomolecules like soluble proteins and hormones, bone EVs could carry more complex information and have certain targeting regulations for other organs or tissues [62]. Studies on senescent EVs give novel perspectives into intra- and interorgan communication in overall aging. Based on these findings, it is clear that senescent EVs have a growing significance as biomarkers for exploring the patterns of aging. Furthermore, inhibiting their release or modifying their composition represents a promising therapeutic approach for age-related diseases. Nonetheless, there are still concerns about the limitations of research on the topic and the continuity of future clinical transformations.

Although senescent EVs are candidates for aging biomarkers, there is still a gap in their clinical application. Early diagnosis is important for specific biomarkers, but senescent EVs have not been proven to be early indicators of most diseases. Since the progression of age-related diseases results from complicated causes, although we have widely discussed senescent EVs and their cargo-mediated pathological mechanisms

within the bone or between the bone and other systems, it is still challenging to determine whether a specific targeting factor from senescent EVs is involved in inflammation, oxidative stress or senescence. Therefore, a single, simple molecule that can cover the early phase of a disease cannot be easily identified. Recently, Basisty et al. [116] defined a senescent proteomic atlas of EVs, termed extracellular vesicle SASP (eSASP). The eSASP factors were isolated from two types of cell lines, which are induced by several kinds of senescence inducers. This may help us develop a resource to identify possible SASP factors associated with aging and related diseases.

Additionally, quite a few current research studies on senescent EVs are still mostly focused on natural molecules, especially miRNAs and their downstream mechanisms. Some avant-garde ideas are needed to get out of this rut. Mitochondrial dysfunction is an important inducer of aging. Recently, mitochondrial EVs have attracted attention, in which mitochondria involved in EVs serve to regulate the metabolism of recipient cells and prevent unnecessary immune activation. A study [117] identified immune cell-derived EVs in healthy control plasma and observed age-dependent reduced mitochondrial content in several EV subpopulations. It has been demonstrated in vitro [118] that healthy human BMSCs transfer MVs containing partially depolarized mitochondria to rescue macrophages from oxidative stress. Macrophages endocytosing these mitochondria were enhanced in oxidative phosphorylation (OXPHOS) and appeared to have an anti-inflammatory phenotype. Although it is still unknown whether and how the intercellular transfer of mitochondria changes in the aged BMME, it might open a new way to explore a novel mechanism against senescence.

With chronological aging, the skeleton represents the internal characteristics of senescence and, at the same time, interrelates with other body systems, which in turn accelerates whole-body aging. In this article, we describe aging-associated molecular, cellular, and systemic changes in bones and mainly focus on the senescent modifications and functions of EVs, which are an important part of the paracrine pathway. Although targeting senescent EVs or their cargoes has great potential in the future to cure age-related diseases, more extensive research is needed on the journey to crystalizing our understanding of their missions in bone aging and overall aging processes.

CRediT authorship contribution statement

Jiahui Shen: Writing – review & editing, Writing – original draft. **Lingling Hu:** Writing – review & editing, Writing – original draft. **Xiaoyuan Huang:** Writing – review & editing. **Jiajie Mao:** Writing – review & editing. **Yuzhu Wu:** Writing – review & editing. **Zhijian Xie:** Writing – review & editing, Supervision, Conceptualization. **Yanhua Lan:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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