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REVIEW

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Extracellular vesicles in age-related diseases: disease pathogenesis, intervention, and biomarker

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Abstract

Aging is a multifactorial biological process characterized by the irreversible accumulation of molecular damage, leading to an increased risk of age-related diseases. With the global prominent rise in aging populations, elucidating the mechanisms underlying the aging process and developing strategies to combat age-related diseases have become a pressing priority. Extracellular vesicles (EVs) have gained significant attention due to their role in intercellular communication. EVs are known for their ability to deliver biocargoes, such as miRNA, proteins, and lipids, implicating their involvement in disease pathogenesis and intervention. In this review article, we explore the dual role of EVs in age-related diseases: contributing to the pathogenesis of diseases by transferring deleterious molecules, while also offering therapeutic ability by transferring beneficial molecules. We also highlight the application of EVs as biomarkers for early diagnosis of age-related diseases, paving the way for early intervention and precision medicine. Additionally, we discuss how analysing the composition of EVs cargo can provide insights into disease progression. Finally, we address the challenges and future perspectives of EV-based-therapy in clinical translation, including standardization of EVs isolation methods and improving cargo specificity.

Highlights

- Extracellular vesicles in physiological and pathological situations are a double-edged sword.
- Omics studies suggest that extracellular vesicles are promising targets for *in vitro* diagnostics.
- Using extracellular vesicles to treat age-related diseases is a highly promising intervention strategy.
- The precise components of extracellular vesicles are crucial for understanding their pathogenic or therapeutic mechanisms.

Keywords Extracellular vesicles, Age-related disease, Pathogenesis, Therapy, Biomarker

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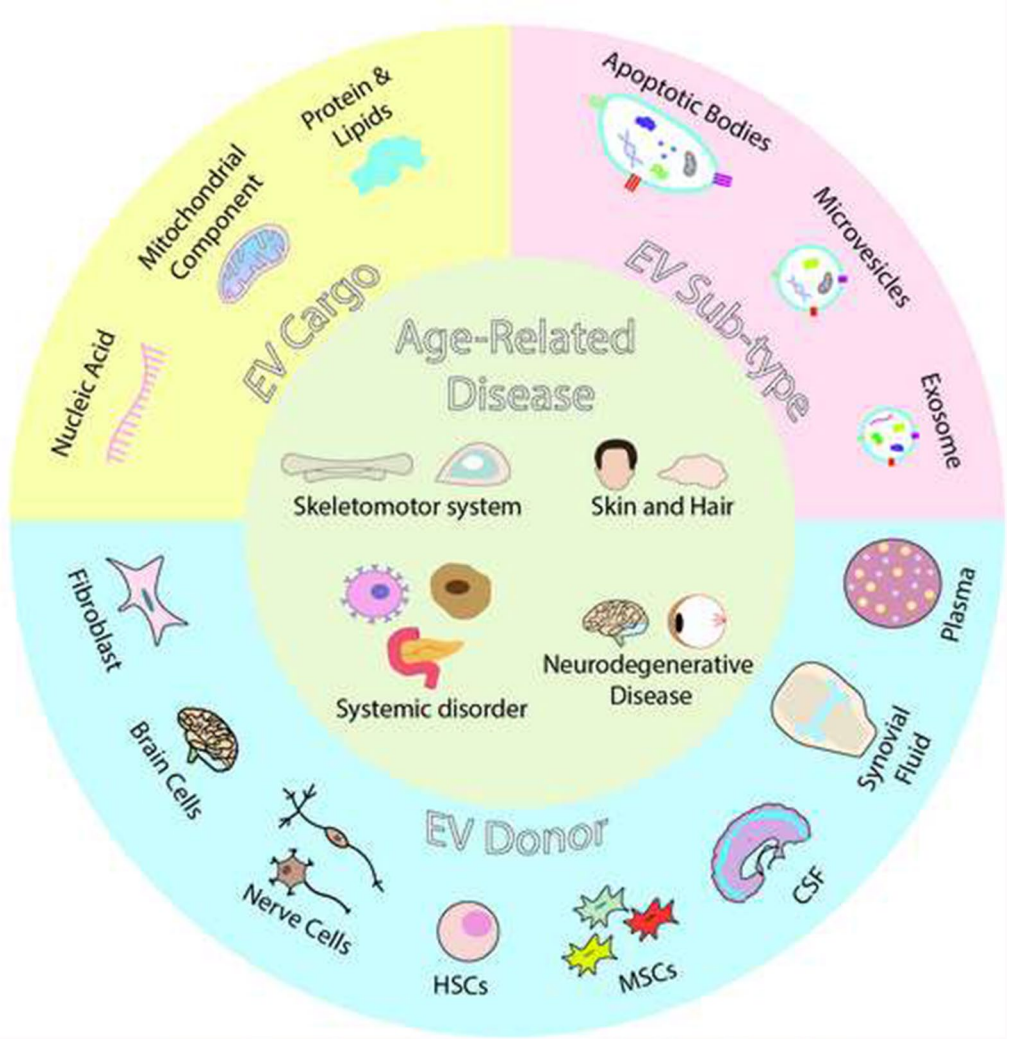
Graphical abstract



Introduction

Aging is an irreversible physiological process which involved significant genetic, transcriptional, translational, epigenetical, and signal transduction alterations [1–3]. These alterations accumulate over time and lead to an increase in disease [4]. According to the data collected by World Health Organization, the number of people aged 60 and above is exceeding the number of children aged below 5 years old, and this number will increase from 12% in 2015 to 22% of world population in 2050 [4]. With this growing aging population, research in the age-related studies becomes a very promising field.

López-Otín et al. [5] proposed 12 aging hallmarks: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macro autophagy, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis. All these hallmarks are interconnected and contribute to the individual aging process. The categorization of these hallmarks leads to a better understanding towards multifactorial pathogenesis of aging process. The progression of aging has been found to be a cause of sEVseral diseases. Approximately 92 diseases have been reported to be associated with the aging process and categorized as age-related diseases [6]. Some of these diseases include skin aging, Parkinson's disease, Alzheimer's diseases, osteoarthritis, osteoporosis,

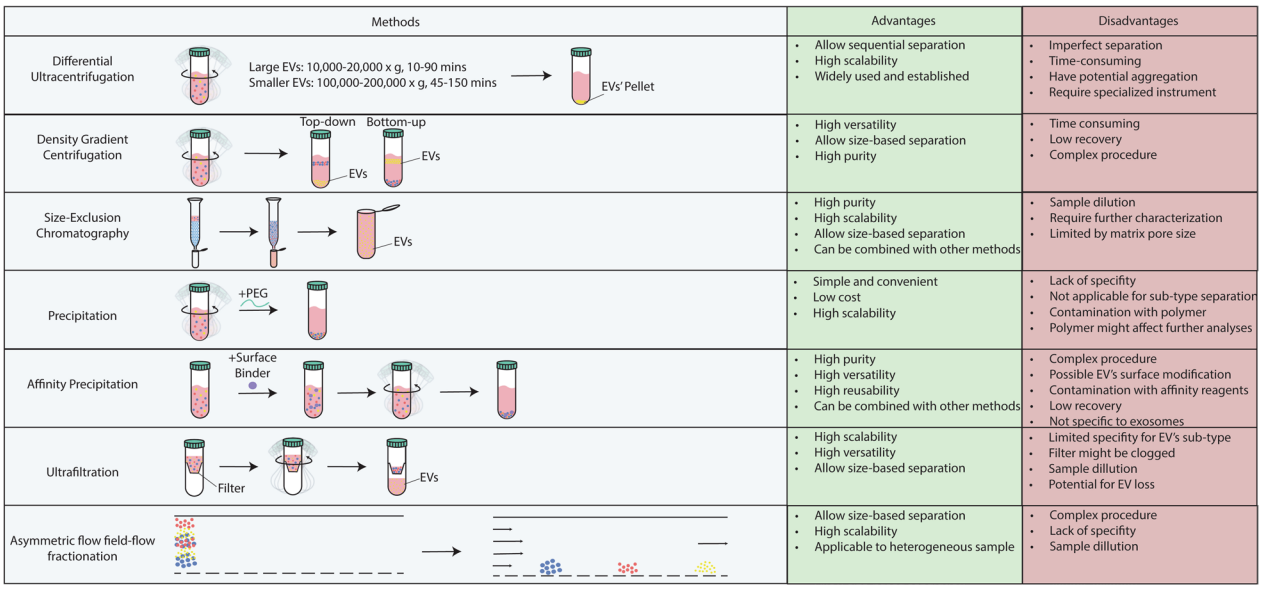


Fig. 1 The purification and concentration of EVs can be categorized into three distinct groups based on their recovery and specificity: high recovery-low specificity (precipitation and ultrafiltration), intermediate recovery-intermediate specificity (size-exclusion chromatography, differential ultracentrifugation, affinity precipitation, and differential gradients), and low recovery-high specificity (asymmetric flow field-flow fractionation) [10, 14]. Currently, there are no standardized methods for purifying EVs from other biomolecules. Consequently, the selection of EV isolation and purification methods should be based on a critical evaluation of the advantages and disadvantages of each technique, while also considering their suitability for subsequent analysis of the EVs. This approach ensures that the chosen method aligns with the specific requirements of the research objectives and the nature of the EVs under investigation

hair disorders, and immunosenescence. The number of patients suffering from age-related diseases is increasing at an alarming rate every year [4]. Thus, it is essential to develop effective treatments to alleviate the burden of age-related diseases.

Extracellular vesicles (EVs) are lipid-bound nanosized structures released from the cells into circulatory system and biological fluids such as breast milk, urine, blood, saliva, amniotic fluid, lymph, and ascites [7–9]. EVs can be isolated from other biomolecules using sEVseral methods, with ultracentrifugation as the most common methods used (Fig. 1). EVs are categorized into three types based on the particle size: exosomes/small EVs (sEVs) (30–150 nm), microvesicles (100–1000 nm), and apoptotic bodies (1–5 μm) [10–13]. Their features can be identified through analysing their protein marker. They are abundant in protein bound plasma membrane (Tetraspanins, Major Histocompatibility Complex (MHC) proteins, and glypicans) and cytosolic proteins (Heat shock protein (HSP), ALIX, Cytoskeleton) [14]. Besides their protein surface marker, these vesicles are well known as carrier for endogenous cargo, including proteins, nucleic acids (microRNA, mRNA, DNA, lncRNA), lipids, organelles (mitochondria, endoplasmic reticulum), and even cytoskeletal components, which involve in biological pathways (Fig. 2) [9, 15, 16]. This function of EVs orchestrates the transcriptional, post-transcriptional, signaling, and material transduction, as well as trans-organelle signal exchange processes [17, 18]. Their features can be identified through.

Recent studies have explored EVs involvement in aging process through cargo transfer, as well as potential biomarker tools (Fig. 3). For instance, in senescent human umbilical vein endothelial cells (HUVECs), EVs released by the cells deliver elevated amount of miR-21-5p and miR-217, which impair the expression of DNA methyltransferase 1 and Sirtuin-1 (SIRT1), thus affecting DNA methylation and cells replication ability [19]. Other studies have also discovered that EVs from aging cells contain reduced amount of mitochondrial DNA (mtDNA), and transferring these EVs to recipient cells results in lower basal and maximal respiration, which aligns with one of the hallmarks of aging: mitochondrial dysfunction [20]. On the other hand, treatment with EVs from younger cells can rejuvenate aging cells, by transferring beneficial molecules. For example, EVs secreted from young mesenchymal stromal cells (MSCs) contain higher amount of autophagy-related mRNA, such as Sirtuin-1/2/3/7 and C-X-C chemokine receptor type 4 (CXCR4) and exhibit anti-aging effects by transferring miR-17 and miR-34a to aged cells [21]. Another study identified antioxidant protein, peroxiredoxins, in EVs secreted by induced

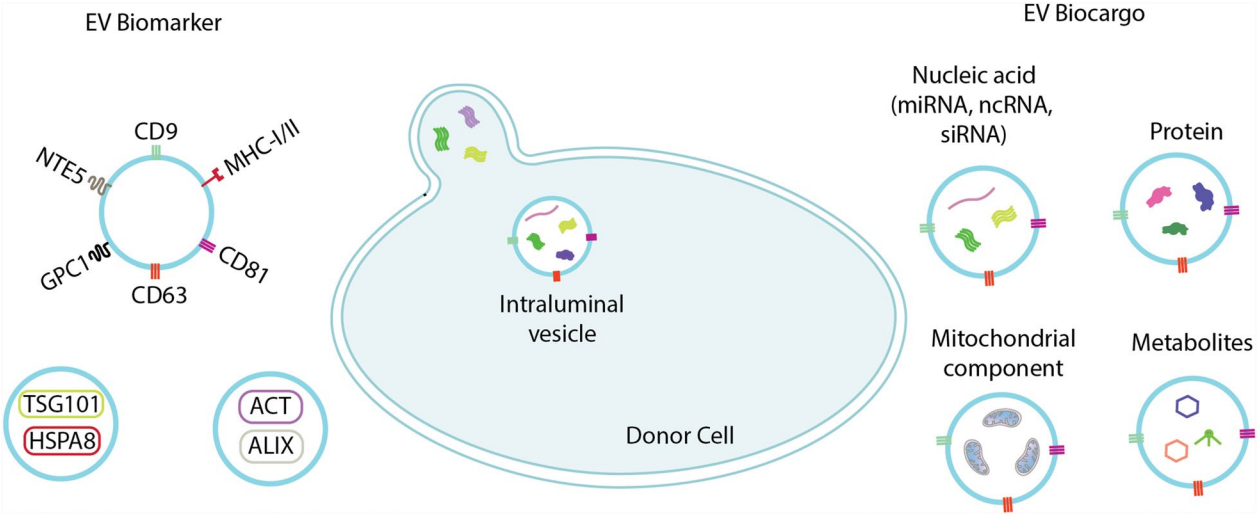


Fig. 2 EVs are lipid-encapsulated membranous structures that are secreted by cells, and they include sEVs, microvesicles, and apoptotic bodies, each characterized by distinct size profiles. SEVS are released into the extracellular space when multivesicular bodies (MVBs) fuse with the plasma membrane, expelling their intraluminal vesicles. In contrast, microvesicles are formed through the direct budding and scission of the plasma membrane. Apoptotic bodies, on the other hand, are cellular fragments derived from apoptotic cells and contain various cellular components [308]. The primary role of EVs is in facilitating cell-to-cell communication, as they transport bioactive cargo, such as nucleic acids, proteins, and mitochondrial remnants, to target cells. EVs can be characterized by their distinct protein markers, which are crucial for their identification and classification. According to the MISEVS2023 guidelines, proteins used to determine the presence of EV features are categorized into two groups. Category I includes proteins that are associated with the plasma membrane, such as tetraspanins, Major Histocompatibility Complex (MHC) proteins, and glypicans. Category II comprises cytosolic proteins that are also found in EVs, including ALIX, TSG101, Heat shock proteins (HSP), and components of the cytoskeleton [14]

pluripotent stem cells (iPSCs), which help in reducing reactive oxygen species (ROS) level and counteracting aging phenotype in aging cells [22]. With these findings, EV-based treatment and diagnosis is expected to be developed and applied to address the burden of age-related disease. Furthermore, by analysing the anomalies in their cargoes, EVs have also emerged as potential biomarker for age-related diseases.

Given the recent advances in EV-based therapeutic approaches, a systematic review of the precise effector molecules and mechanisms of EVs is therefore warranted. Hence, in this review, we will dissect and pinpoint the mechanisms and endogenous cargo within EVs that are involved in age-related diseases, as well as the challenges and future prospects for EV-based therapy for these diseases.

Extracellular vesicles' role in age-related diseases

Age-related diseases, which include conditions like skin aging, hair loss, neurodegenerative disorders, skeletomotor disorders, immunosenescence, cancer and metabolic disorders (Fig. 4), stem from the 12 hallmarks of aging identified by López-Otín et al. [5]. These diseases are becoming increasingly significant with the growing aging population. EVs, as emerging nanomolecules, have been under spotlight for their role in the pathophysiology of aging and age-related diseases. EVs, capable of carrying nucleic acids, organelles, proteins, lipids, and other biomolecules, are implicated in the development of these conditions (Fig. 5). In this article, we will delve deeper into the mechanisms of EVs in age-related diseases, their potential as therapeutic interventions, and their significance as biomarkers, offering insights into the complex interplay of EVs in the aging process (Fig. 6).

Skin and hair

Skin aging

The skin is the largest organ of the human body and serves as the primary barrier against external stimuli, such as illumination, pathogens, and physical and chemical injury [23, 24]. It is composed of three layers: the epidermis, dermis, and subcutaneous tissue. The epidermis is the outer-most layer composed of several cell types, including keratinocyte and melanocyte [25]. Keratinocytes are a crucial component of the epidermis, as they construct the epidermis structure. In contrast, melanocytes produce pigment, impart colour to the skin, and provide protection from ultraviolet (UV) light [26]. The dermis is the second layer of the skin, containing extracellular matrix (ECM) components such as collagen and elastin fibres, which maintain skin

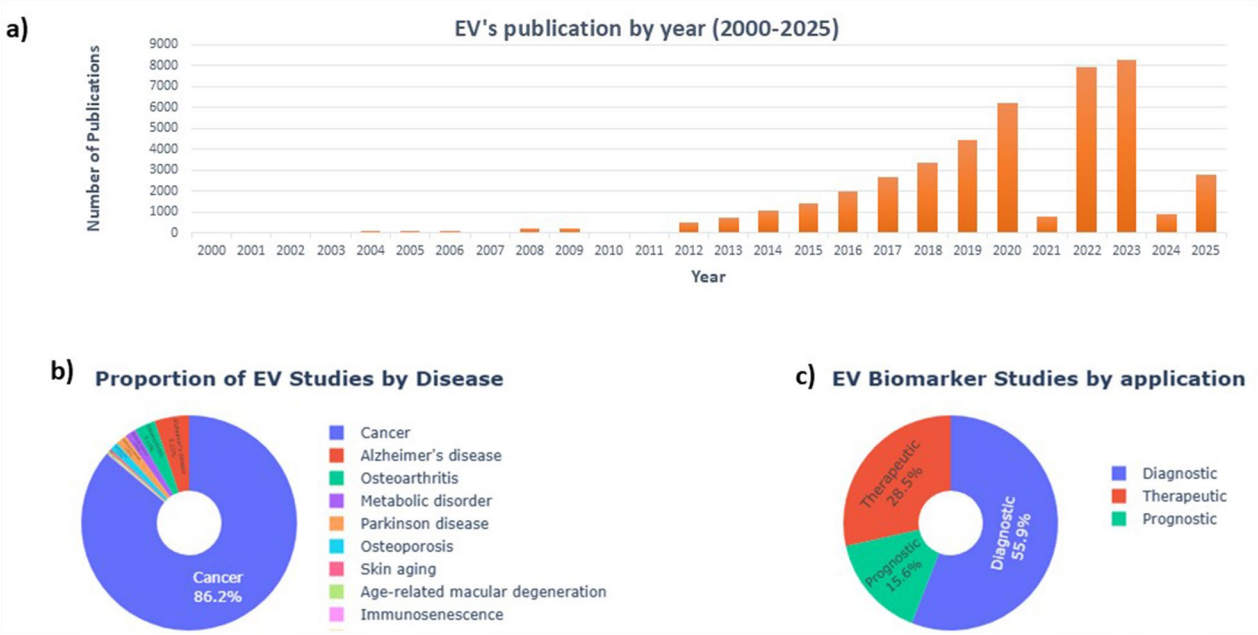


Fig. 3 Trends on EV studies. **a** EV's publication from 2000 to 2025, retrieved from Pubmed with keywords of "Extracelluler vesicles", "Exosomes", "Microvesicles", "Ectosomes". **b** Proportion of EV studies in age-related disease (Excluding review article), data retrieved from Pubmed with keywords "Extracellular vesiscle" and "Disease name". **c** EV Studies as biomarker categorized by application (Excluding review article), data retrieved from Pubmed using keywords "Extracellular vesicles" and "Biomarker" and "Diagnostic/Therapeutic/Prognostic"

elasticity [25]. However, during aging process, oxidative stress, autophagy inactivation, and the upregulation of matrix metalloproteinase (MMP) lead into a decrease in keratinocytes and melanocytes proliferation. Concurrently, the aging process in the dermis layer results in increased ECM protein degradation. Visually, this will make the skin appear thin, wrinkled, saggy, and exhibit variations in colour [27].

Oxidative stress is central to collagen degradation. With age, the accumulation of reactive oxygen species (ROS) activates mitogen-activated protein kinase/nuclear factor kappa beta (MAPK/NF-kB) pathway through TNF-α stimulation, leading to the production of matrix metalloproteinases (MMPs) [28]. MMPs are a family of zinc-dependent endopeptidases that capable of degrading the extracellular matrix (ECM), including collagen [29, 30]. Recent studies found that UVA radiation increases the expression of Cathepsin K through the activation of MAPK pathway in human dermal fibroblasts, which degrades elastin and induces solar elastosis [31]. Additionally, senescent cells that accumulate with age will secrete senescence-associated secretory phenotype (SASP) factors, which have found to affect surrounding normal cells [32]. Types of senescence-associated secretory phenotype (SASP) include pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)−1, IL-6, IL-18, and interferons (IFN) [33], as well as various MMPs [32]. These factors are involved in skin aging, especially their elevated production levels will result in elevated collagen degradation and enhanced expression of MMP genes [33].

The pathogenic role of EVs in skin aging In the deleterious aspect, senescent cells might secrete EVs that differ from those derived from normal cells. It was found that senescent fibroblasts are capable of secreting high levels of EVs, that occurred due to the increasing activity of neutral sphingomyelinase (nSMase), oxidative stress, and altered lysosomal activity in the senescent cells [34]. nSMase is an enzyme that catalyses the cleavage of sphingomyelin into ceramide and phosphocholine, which are important building blocks of the epidermis structure [35]. However, this increased activity of nSMase induced by the presence of TNF-α may cause skin inflammation [36]. Furthermore, it has also been found that the senescent fibroblasts-derived EVs highly express the proinflammatory cytokine IL-6 and become less supportive in keratinocyte differentiation and barrier function. The diffusion of senescent cell-derived EVs might influence normal cells and push them into premature senescence state. Exosomes derived from senescent endothelial cells containing miR-767 were observed to regulate the fibroblast aging through the suppression of the transforming growth factor β-activated kinase 1 binding protein 1 (TAB1) gene [37]. TAB1 is a protein that

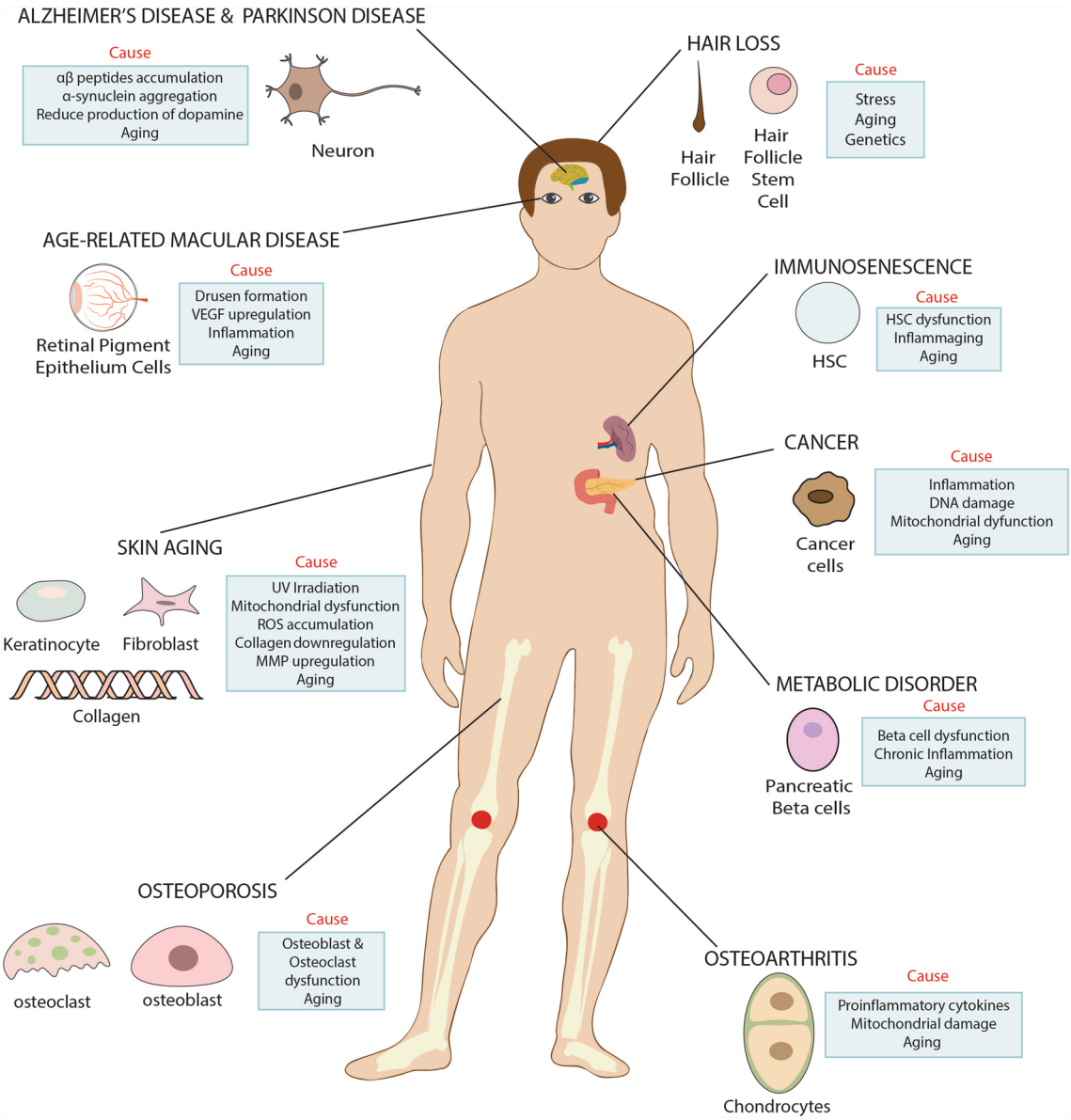


Fig. 4 The primary instigators and involved cells of different age-related diseases. Aging and aging-associated factors, such as inflammation and mitochondrial dysfunction, are primary contributors to various cell types disruption, including neurons, hematopoietic stem cells (HSCs), pancreatic beta cells, retinal pigment epithelium (RPE) cells, hair follicles, chondrocytes, osteoblasts, and fibroblasts, leading to the manifestation of age-related disorders

binds to transforming growth factor-β (TGF-β)-activated kinase 1 (TAK1), thereby regulating cell death. The role of TAB1 is to activate TAK1 in response to osmotic stress [38]. By inhibiting TAB1 through miR-767 target binding, TAK1 cannot be activated, leading to cell senescence and apoptosis, also suppressing cell proliferation.

EVs as therapeutic tools for skin aging In the beneficial aspect, EV-derived stem cells carry regenerated compounds from their donor cells, and sEVseral studies have proven their potentials. For example, sEVss derived from adipose-derived stem cell (ADSC) contain overexpressed miR-1246. In vitro studies demonstrated that miR-1246 transferred by sEVss can bind to Glycogen synthase kinase 3β (GSK3β), an autophagy inhibitor. In a UVB-induced photoaging mouse model, treatment using miR-1246-overexpressed sEVss significantly decrease the expression of GSK3β and p62 while elevating the level of light chain 3 II (LC3II) and inducing autophagy flux in the skin. Additionally, the ROS, MMP-1, and DNA damage

were reversed to improve the cell viability. Procollagen type I level is increased, contributing to tissue elasticity [39]. Beside miRNA, 14-3-3ζ protein delivered by sEVs derived from human umbilical cord MSC (hucMSC) exhibited similar results in attenuating skin photoaging. Through SIRT1-dependent pathway activation, in vitro treatment of UV-irradiated human keratinocyte line (HaCat) cells with the hucMSC-derived sEVs showed an increase ratio of LC3II/I and autophagy activity, while apoptosis was significantly reduced [40]. Autophagy is a cellular metabolic process that removes damaged organelles and molecules, and alleviates stress caused by infection and pathology [41], but the activity of autophagy declines during aging [42]. Therefore, decreased autophagy is a sign of the loss of proteostasis, which occurs in aged organisms [43]. Therefore, maintaining autophagy activity is an important aspect for skin aging treatment.

Another cargo carried by EVs, besides miRNA, is noncoding RNA. Latest studies demonstrated how treatment with overexpressed long noncoding RNA H19 (H19) in ADSC-derived sEVs (H19-Exo) may ameliorate photoaging in skin. Bioinformatics analysis conducted found that H19 could target bind with miR-138. Treatment with H19-Exo decreases the miR-138 expression, enhancing SIRT1 expression in both in vitro and in vivo studies, that subsequently inhibiting MMP production and elevating collagen type I synthesis [44]. Previous studies found that target genes of miR-138 are p63 and SIRT1 [45]. SIRT1 is well defined to have the ability to delay cellular senescence [46], suggesting that miR-138-SIRT1 axis may

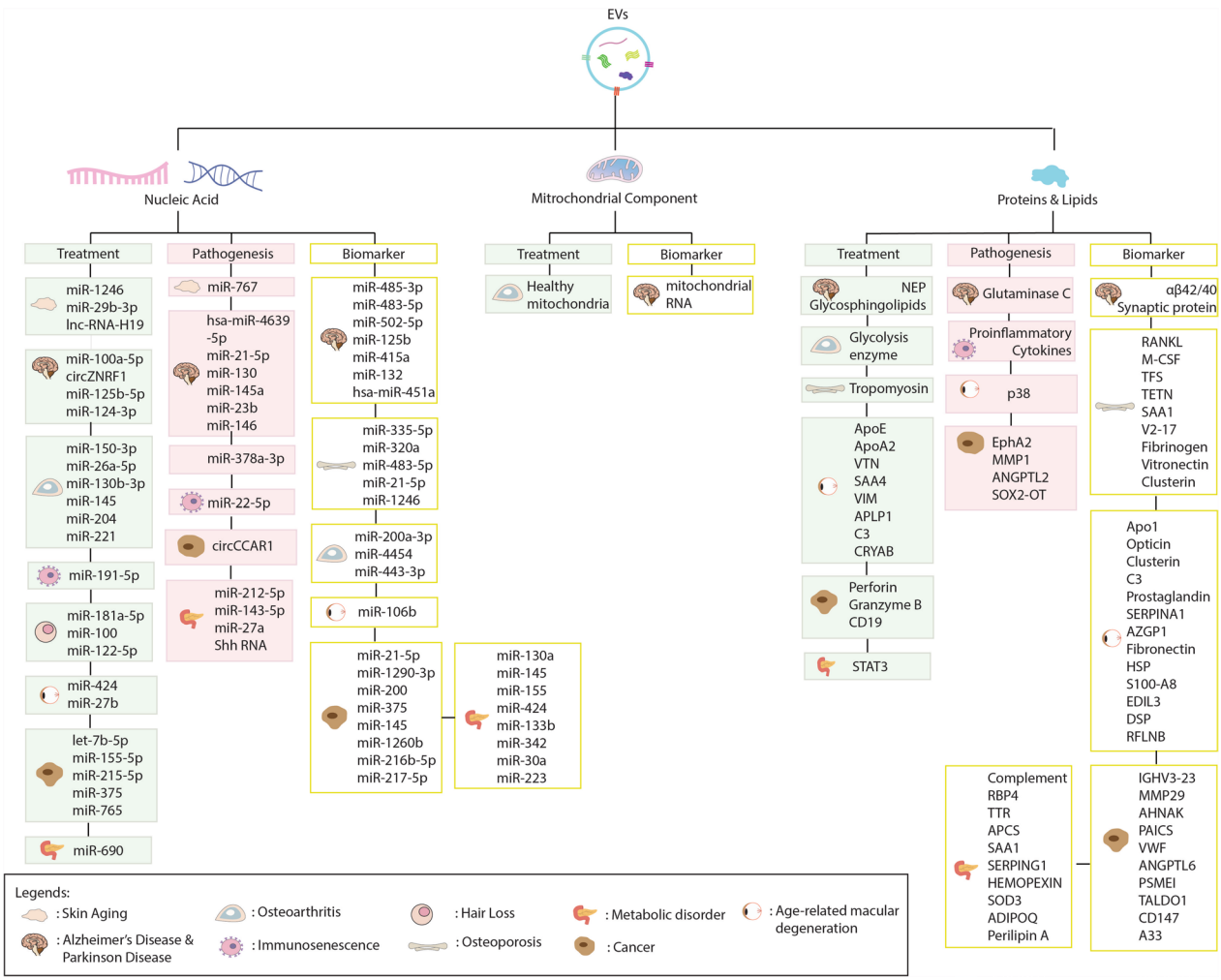


Fig. 5 EVs play an integral role in the progression and potential therapeutic intervention of age-related diseases. They transport specific cargo, including nucleic acids, proteins, and lipids, which can modulate cellular pathways regulating neurodegenerative diseases (AD, PD, and AMD), skin aging, hair loss, skeletomotor system (OA and OP), and Systemic disorder (immunosenescence, cancer, and metabolic disorder), highlighting the role of EVs in both disease pathology and treatment strategies

offer new anti-aging targets. Additionally, bone marrow-derived MSC sEVs (BMSC-Exo) were found to carry miR-29b-3p and significantly alter the negative effect of UVB-irradiation in the models. Treatment using BMSC-Exo to the human dermal fibroblasts (HDFs) model showed significant improvement in type I collagen synthesis after UVB-irradiation. By targeting MMP2, miR-29-3p-contained EVs suppress the expression of MMP1 and MMP-3 mRNA [47].

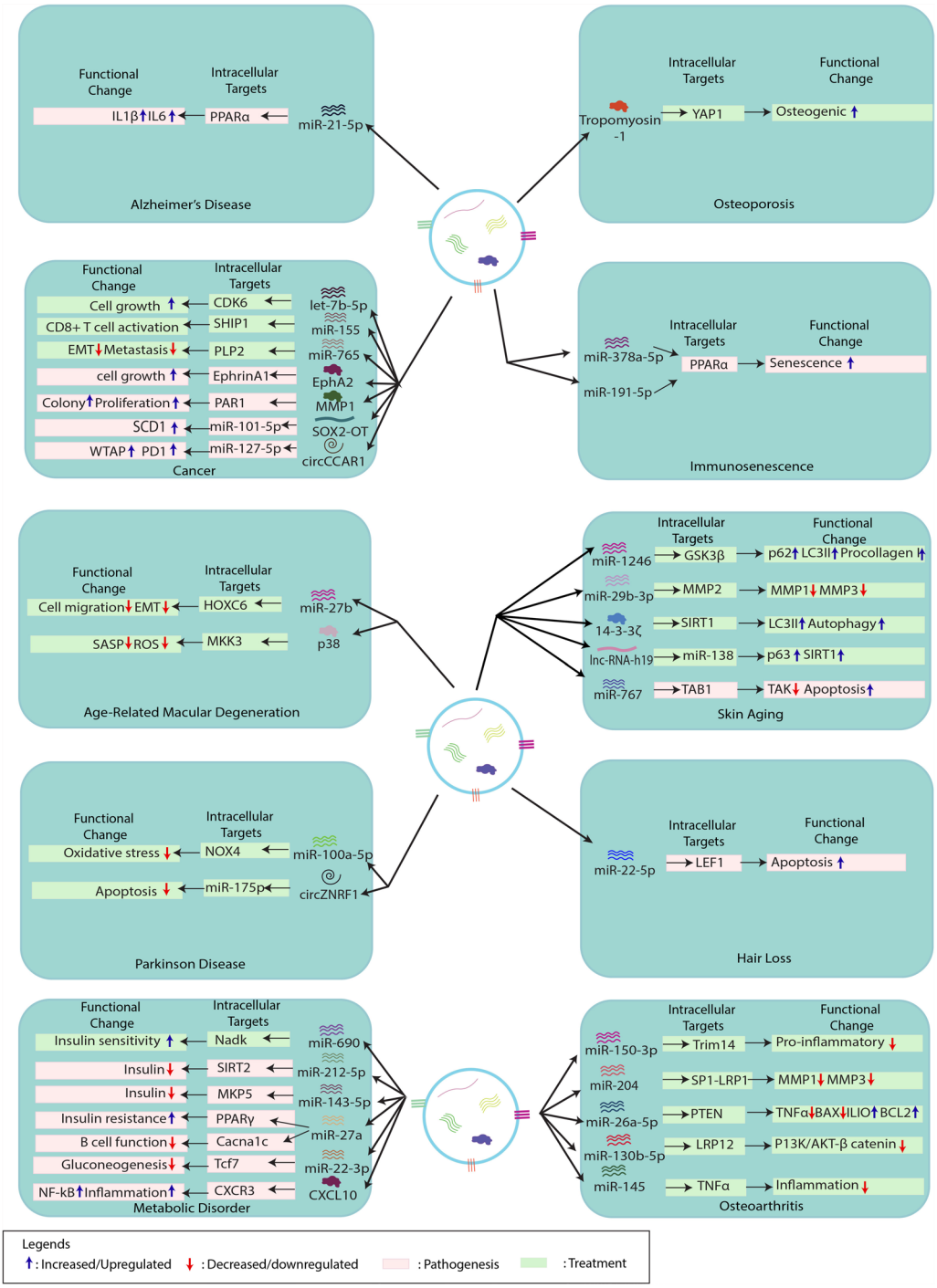


Fig. 6 Complex interplay of EVs. EVs, through their cargo transfer capabilities, interact with intracellular proteins, influencing cell function. Depending on the molecules they carry and interact with, EVs can either disrupt or restore cellular homeostasis, highlighting their dual role in disease progression and potential therapeutic applications

Androgenic alopecia (AGA)

Hair is undoubtedly one of the most important aspects influencing an individual's appearance. However, environmental factors, stress, ageing, and genetics might lead to hair loss. AGA is the most common type of alopecia (hair loss), affecting over 50% of elderly men and 15% of postmenopausal women [48, 49]. Currently, the FDA has approved only two medical treatments for AGA: topical minoxidil and oral finasteride [50]. However, minoxidil could cause allergic reaction in some individuals [51] and long-term usage of finasteride may lead to reduced sex drive, depression, breast lumps, and in some cases, allergic reactions [52]. Therefore, there is an urgent need for alternative treatment options without these unwanted side effects.

EVs as therapeutic tools for AGA Several signalling pathways regulate hair growth, including sonic hedgehog (SHH), TGFβ, NOTCH and Wnt/β-catenin pathways. The Wnt/β-catenin play a central role in the hair cycle by inhibiting glycogen synthase kinase 3β (GSK3β), particularly during anagen phase [53]. It was discovered that sEVs derived from dermal sheath cup cells (DSCCs), a specific subset of hair follicle mesenchymal stem cells, can reverse H_2O_2-induced or age-generated dermal papilla cell aging and activate Wnt/β-catenin signalling pathway, contributing to hair growth in senile alopecia [54]. Additionally, ADSC-derived sEVs exhibit similar effects to DSCC-derived sEVs, inducing the expression of Ser9 phosphorylation of GSK3β and facilitating the nuclear translocation of β-catenin, thus activating the Wnt/β-catenin signalling pathway [55].

Several studies have explored the ability of EV-encapsulated miRNAs to induce hair growth by remodelling Wnt/β-catenin pathway in hair follicle cells. For instance, dermal papilla cell sEVss-derived miR-181a-5p [56] and ReNcell VM cell-derived miR-100 have been investigated (ReNcell VM cell is a neural progenitor cell line) [57]. In contrast, miR-22-5p suppresses hair growth by targeting LEF1, a crucial transcriptional factor in the Wnt signalling pathway. The sponging of LEF1 by miR-22-5P inhibits the activation of the Wnt/β-catenin signalling pathway, leading to hair follicle cell apoptosis [58]. In addition to the Wnt/β-catenin pathway, the TGFβ1 pathway also regulates hair growth [53]. Mir-122-5p carried by ADSC-derived sEVs, shows therapeutic potential for AGA by counteracting DHT-induced blockage of hair follicle activation through the TGFβ/SMAD3 signalling pathway [59].

Although several studies have successfully demonstrated EV-based treatment for AGA, the exact mechanism of exosomes-mediated therapy remain unclear. Many studies have focused on the elucidation of EV-miRNAs, often neglecting other functional molecules, such as proteins and metabolites, resulting in incomplete answers to critical questions about mechanisms and effector molecules. Further research is needed to unlock the molecules involved in exosomes-mediated hair growth and to develop targeted therapies for AGA.

Neurodegenerative disorders

A major challenge in neurodegenerative diseases treatment is the transport of drugs and drug delivery systems across blood–brain barrier (BBB), a diffusion barrier that acts as a shield and interferes with the inflow of certain compounds from bloodstream to the brain [60]. EVs possess advantages due to their small size, low immunogenicity, and prolonged half-life. Especially, stem cell-derived EVs have been found to mediate in both pathogenesis and treatment of central nervous system (CNS) diseases. Most of stem-cell derived EV were found to able to cross the BBB to mediate in the CNS disease pathogenesis and treatment [61]. Therefore, in recent years, studies have been focused on elucidating the role of EV in neurodegenerative diseases.

Alzheimer disease (AD)

Some population suffer from dementia that often characterized by symptoms such as memory loss, disorientation, difficulty in performing daily activities, personality changes, and inappropriate behaviour. These symptoms are caused by factors such as aging, depression, smoking, obesity, social isolation, hypertension, and diabetes [62]. In 2020, it was estimated that about 55 million people were diagnosed with dementia globally, mainly affecting individuals aged over 65, thereby causing a huge socioeconomic burden [63]. Moreover, recent study shows that 76.3 and 143 out of 100,000 people in the age groups of 30–64 years old and 45–64 years old, respectively, are at risk for dementia [64]. This suggest that dementia may also affect younger individuals, causing a greater burden due to potential delays in diagnosis and misdiagnosis [65]. Alzheimer's disease is the most common type of dementia, with approximately take up 60–70% of dementia cases [62].

The pathogenic role of EVs in AD Alzheimer's disease occurs as a result of the accumulation of amyloid-beta (Aβ) peptides in the medial temporal lobe and neocortical structures of the brain [66]. This accumulation followed by oligomerization induce phosphorylated tau in neurons, leading to the formation of neurofibrillary tangles (NFT), neuroinflammations, and impaired neural activity [67]. EVs released by brain cells may mediate Aβ pathogenic propagation, as they have found to contain these peptides. However, the mechanisms on how EVs mediate

in AD pathogenesis development is not elusively understood. Recent studies have attempted to elucidate these mechanisms [68]. They found that EVs derived from cerebrospinal fluid (CSF) of AD patient contain high expression of Aβ42/40. When these EVs transfer their cargo into recipient neurons, the neurons show an accumulation of thioflavin S-reactive protein aggregates, leading to impairments in Ca^{2+} signaling and mitochondrial dysfunction [68].

Neuroinflammation is attributed as a central mechanism of AD pathogenesis [69]. Microglia, blood-derived mononuclear cells and astroglia are three functional cellular players in neuroinflammation microenvironment. Microglia become very sensitive to inflammatory stimuli during pathological aging and other triggers. By binding soluble amyloid β (Aβ) oligomers and Aβ fibrils via receptors including class A scavenger receptor A1, CD36 and toll like receptors, microglia start to engulf Aβ fibrils by phagocytosis and activate innate immune response. In aged mice, rats and primates, the transcripts about microbe recognition and host defence were upregulated, leading the abnormal microglia sensitivity to inflammatory stimuli [70]. The microglia sustained activation is leading to inflammatory AD pathogenesis by producing excessive amounts of pro-inflammatory cytokines (i.e., pro-IL-1 β, IL-6, TNF-α), macrophage inflammatory peptide (MIP-1α) and macrophage colony-stimulating factor (M-CSF) [71]. In vitro and traumatic brain injury in vivo tests showed that exosomes containing miR-21-5p from PC12 neuron cells were phagocytosed by microglia and induced M1-type microglia polarization, increasing in the expression of IL-1B and IL-6, as well as the accumulation of p-tau [72]. And this exosome promoted the apoptosis of neuron cells and formed cyclic cumulative damage which may progress into AD [72]. MiR-21-5p is known to target PPARa and impair the astroglial uptake and degradation of Aβ, as well as activation of nuclear factor-kappa B and activator protein-1 pathways patways through to PPARa ligands [73, 74]. In addition to microglia priming, the presence of glutaminase in the brain can also induce neuroinflammation, leading to AD progression. High expression of glutaminase C (GAC) can be found in early AD mice model [75]. GAC can influence the polarization of microglia into pro-inflammatory phenotype. Furthermore, the microglial-derived exosomes were observed to contain higher amounts of GAC than controls. Injection of these exosomes into rat microglia caused an increased pro-inflammatory cytokines expression after 24 h post-injection. This GAC-rich sEV also carries classic pro-inflammatory miRNAs, such as *miR-130*, *miR-145a*, *miR-23b*, and *miR-146a* [75]. For example, miR-145a itself have been associated with P13 K/AKT pathway, a pathway that are well-known to induce AD pathogenesis [76]. These findings emphasize how exosomes participate in disease progression through neuroinflammation.

EVs as biomarker for AD diagnosis Furthermore, EVs also serve as biomarkers for AD pathogenesis, improving early diagnosis of AD, especially in non-symptomatic stage. It was discovered that saliva-derived EVs from AD patients contain higher expression of miRNA-485-3p compared to healthy controls [77]. This brain-enriched miRNA is detectable in brain tissues, CSF, and plasma, with its expression in AD patients positively correlating with the degree of Aβ plaque formation [78]. Additionally, miR-125b-1-3p and hsa-miR-451a are differentially expressed in sEVs from AD patients'peripheral blood, being more abundant than in healthy individuals. These miRNAs are implicated in neuronal damage; miR-125 downregulates 15-lipoxygenase, while miR-415a suppresses expression levels of activating transcription factor 2 (ATF2), leading to neuronal damage and apoptosis [79]. EVs from individuals with mild cognitive impairment (MCI) also showed higher expression levels of miR-483-5p, miR-502-5p, and miR-132-3p [80]. In addition, other studies reported a significant correlation between EV-enriched miRNA-1290 and medial temporal atrophy, a biomarker of AD [81]. Furthermore, exosomal levels of synaptic protein such as growth-associated protein 43 (GAP43), synaptotagmin 1, and synaptosome associated protein 25 (SNAP25) are decreased, contrasting with elevated levels in CSF [80, 82]. These proteins serve as important biomarker for AD, although further studies are needed to explain their specific roles in AD pathogenesis [83]. EVs also contain mitochondrial components. EVs derived from AD sample shows signs of mitochondrial abnormalities, including lower levels of complexes I, III, IV, and V of ATP synthase, superoxide dismutase 1 (SOD1), and catalytic activity of complex IV, indicating abnormalities in electron transport chain (ETC) that may leads into ROS accumulation, triggering αβ aggregates [84]. An elevation of mtRNA expression in EVs might also be a result from mitochondrial damage in brain cells due to Aβ and τ aggregations in AD. EVs secreted from astrocytes, microglia, and neurons cultured with exposure to αβ aggregates and H_2O_2 to mimic AD pathogenesis contained elevated amount of mtRNA. Additionally, EVs isolated from AD individuals' plasma serum showed upregulated levels of mtRNA compared to healthy controls [85]. As mitochondrial dysfunction is one of the hallmarks of AD, observing mitochondria in EVs may allow us to detect AD pathogenesis [86].

EVs as therapeutic tools for AD Recent research highlights the potential of extracellular vesicles (EVs) in mitigating Alzheimer's disease (AD) by reducing amyloid-

beta (Aβ) levels and modulating neuroinflammation. Small extracellular vesicles (sEVs) isolated from murine neuroblastoma Neuro2a (N2a) cells have been found to contain glycosphingolipids on their surface, which facilitate the binding of monomeric Aβ peptides and promote their transport to microglia for degradation. This process leads to a reduction in Aβ levels, amyloid deposition, and Aβ-induced synaptotoxicity in the hippocampus of AD mouse models [69].

Additionally, sEVs derived from adipose-derived mesenchymal stem cells (ADSCs) and bone marrow-derived mesenchymal stem cells (BMSCs) have been found to be enriched with neutral endopeptidase (NEP), an enzyme involved in the degradation of Aβ peptides in the brain. Treatment of N2a cells with these sEVs significantly reduced both extracellular and intracellular Aβ levels. Notably, ADSC-derived sEVs exhibit higher NEP levels than BMSC-derived sEVs, suggesting that ADSCs may offer greater therapeutic potential for AD [70].

Beyond Aβ clearance, EVs also play a role in regulating neuroinflammation—a key factor in AD pathogenesis. sEVs derived from BMSCs preconditioned with TNFα and IFN-γ have been shown to induce M2 macrophage polarization in vitro, reduce microglial activation, and enhance dendritic spine density in AD mouse models [71]. These findings suggest that preconditioned BMSC-derived sEVs may not only reduce neuroinflammation but also protect against dendritic spine loss, which is critical for maintaining synaptic integrity. However, the precise molecular mechanisms underlying these therapeutic effects remain to be fully elucidated.

EVs derived from neural stem cells (NSCs) have also demonstrated neuroprotective properties in AD models [87]. Treatment with NSC-derived EVs effectively reduced the expression of pro-inflammatory cytokines IFN-γ and IL-17. Concurrently, these EVs increased levels of the anti-inflammatory cytokine IL-10 and enhanced the presence of $CD19^{+}CD5^{+}CD43^{+}B1$ cells, which play a crucial role in immunoglobulin M (IgM) and IgG balance in neuroinflammation [88]. MicroRNA profiling of this EV identified three key miRNAs, miR-125b-5p, miR-124-3p, and miR-125a-5p, which may contribute to its neuroprotective effects [87]. Among them, miR-124-3p has been previously implicated in neuroinflammation suppression following traumatic brain injury [89–91]. Meanwhile, the miR-125 family has been shown to regulate immune system development, protect B cells from apoptosis [92], and influence synaptic structure in neurons [93].

Parkinson disease (PD)

PD is the second most common neurodegenerative disorder after Alzheimer's disease and is associated with the aging process. It was reported that in 2019, there were 8.5 million cases of PD globally. This number has doubled over the last 25 years and cause significant caregiving burden for individuals, families, and society [94]. The exact cause of PD remains unclear, and recent observations suggest that PD may manifest as a result of several factors [95]. Most patients with PD show symptoms such as bradykinesia, rigidity, tremor, and gait imbalance over a long period of time. However, each individual develops unique PD symptoms, making early detection particularly challenging [96, 97].

Although several factors may contribute to PD, the most prominent symptom occurs when nerve cells in basal ganglia area of the brain are damaged or die, resulting in reduction of dopamine production. While the exact cause of this cellular damage remains unclear, most PD patients experience neuroinflammation and mitochondrial dysfunction [95, 98]. Under infection and injury conditions, brain microglia serve as the first line of defense and protection [99, 100]. M1 microglia secrete pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, IFN-γ, Nitric oxide, along with chemokines like C–C motif ligand-2 (CCL2). Subsequently, M2 microglia secrete anti-inflammatory cytokines such as IL-10 and TGF-β to promote tissue regeneration, repair, and cellular homeostasis [101]. However, in the presence of protein aggregates such as α-synuclein (α-syn), M1 microglia produce excessive ROS and nitric oxide due to the upregulation of major histocompability complex (MHC) II, leading to apoptosis and the inhibition of NF-kB isoforms and C-X-C chemokine receptor 4 (CXCR4), which contribute to PD pathogenesis [100, 101]. In mouse models, depletion of Nf-kB/c-Rel proteins has been linked to the death of dopaminergic neurons and increased α-syn aggregation in substantia nigra [102].

Astrocytes also contribute to PD pathogenesis. Their primary function includes amplifying the immune response alongside microglia, encapsulating neuronal synapses, and controlling BBB permeability. Astrocytes can also polarize into pro-inflammatory A1 and anti-inflammatory A2 phenotypes. In PD, astrocytes can endocytose excessive α-syn released by neuron and transfer it to other cells, thereby spreading the pathogen [103]. The uptake of α-syn by astrocytes can also shift their phenotype into A1 type, leading to excessive secretion of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-18), colony stimulating factors 1 (CSF-1), CSF-2, CSF-3, and chemokines (CCL3, CCL5, CCL7), which can induce dopaminergic neuron death [104].

Alpha-synuclein (α-syn) is a protein located in presynaptic terminal of neurons, where it interacts with protein involved in the reuptake and release of neurotransmitters [105, 106]. It is a key component of Lewy Bodies, which

are one of the hallmarks of PD. Researchers reported that α-syn aggregation may be a contributing factor in the development of PD [95]. The aggregation of α-syn results from modifications to its conformation, creating a toxic beta-sheet conformation [96]. Several potential explanations exist, including mutations in genes such as PINK1 and Parkin, which lead to mitochondrial dysfunction, causing the accumulation of ROS and reducing lysosomal activity for the α-syn degradation [95]. Studies on *C. elegans* have shown that mutations in the Parkin gene result in the accumulation of mitochondria with abnormal morphology, which consume more oxygen than normal mitochondria. Meanwhile, mutations in PINK1 do not cause morphological changes, but still result in mitochondrial accumulation. Since the total oxygen consumption of the mutant PINK1 worms is comparable to that of control group, it can be concluded that each mitochondrion consumes less oxygen. Subsequent observations concluded that the two mutant gene worms experience higher neuronal cell loss [107].

The pathogenic role of EVs in PD In recent years, the propagation of α-syn has been referred to as neurodegenerative synucleinopathies. It was found that the neurodegenerative synucleinopathies may accelerate the progression of PD [108]. In a healthy brain, microglia perform the removal of mutated α-syn. However, mutated α-syn can stimulate microglia to release sEVs containing proinflammatory cytokines as well as the mutated α-syn itself [109]. Additionally, Guo et al. confirmed the presence of α-syn oligomers in $CD11b^+$ exosomes in the CSF of PD patients. These exosomes carry α-syn, inducing protein aggregation in recipient neurons. It was found that treatment with preformed α-syn fibrils stimulates microglia and upregulates Pellino 1, an E3 ubiquitin ligase, which leads to the degradation of lysosomal-associated membrane protein 2 (LAMP2) and then inhibits autophagosome-lysosome autophagic flux in microglia. As a result, the level of exosomes-released from microglia increases, and the amount of α-syn contained within these exosomes also rises. Besides, activated microglia have also been found to release other molecules including proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β [110]. Proinflammatory cytokines also play a significant role in enhancing α-syn aggregation. Furthermore, α-syn present in the blood could transverse into the CNS mediated by EVs, due to its ability to cross the BBB. Healthy iPSC-derived dopaminergic neurons tend to uptake exosomes pretreated with α-syn preformed fibrils, leading to increase amount of α-syn released into the CNS [111]. Along with α-syn, miRNA is another type of pathogenic biomolecule carried by the EVs. Hsa-miR-4639-5p, a miRNA that negatively affects DJ-1, also plays a role in PD pathogenesis [112]. DJ-1 protects the cell during oxidative stress by dissociating from a dimer into a monomer structure, which further translocates into the nucleus, regulating various signalling pathways [113]. However, mutant DJ-1 reportedly cause early onset of PD. Substitutions of amino acids (e.g. L166P) in the DJ-1 structure may disrupt its protein stability and ability to form homodimer, enhancing DJ-1 degradation [114]. Downregulation of DJ-1 results in increased cellular sensitivity towards neurotoxins, leading to the manifestation of PD symptoms [115]. In the present study, it is demonstrated that CNS-derived exosomes play a vital role in elevating plasma levels hsa-miR-4639-5p in PD patients, and a polymorphism in the core promoter elevates hsa-miR-4639-5p expression, eventually amplifying PD risk [112]. These findings suggest that blocking the transmission of pathogenic biomolecule-containing exosomes has the potential for symptomatic relief and may halt the PD progression.

EVs as therapeutic tools for PD The therapeutic potential of EVs in mitigating Parkinson's disease (PD) has been explored, leveraging their size advantage to cross the BBB. Trophoblast stage-derived mesenchymal stem cells (T-MSCs)-sEVs enhance antioxidant capabilities, thereby protecting dopaminergic neuron in MPTP-induced PD mice. Further analysis through small RNA sequencing revealed that these sEVs are enriched with miR-100a-5p, which targets the 3' untranslated region (UTR) of nicotinamide adenine dinucleotide phosphate hydrogen oxidase 4 (NOX4). This targeting mitigates oxidative stress via NOX4-ROS-NRF2 axis [116].

The Bcl2 family, known for its role in apoptosis regulation, has also been implicated in dopaminergic neuronal apoptosis in PD [117]. Knockdown of Bcl2 family proteins in neurons is hypothesized as a potential therapeutic strategy for PD. Treatment of paraquat-induced neuronal cells with miroglia-derived exosomal circ-ZNRF1 can ameliorate neuronal cell apoptosis. Bioinformatic analysis revealed that the exosomal circZNRF1 treatment act as a sponge for miR-17-5p and regulates the binding of Bcl2, thereby reducing cell apoptosis [118].

Age-related cellular stress is a major cause of damage to the nervous system, and EVs derived from stem cells or normal nerve cells can effectively improve cellular stress. For example, neurons treated with oligodendrocyte-derived sEVs exhibit reduced mitochondrial stress and increase bioenergetic metabolism, attributed to the delivery of SIRT2 from mature oligodendrocyte [119]. Despite the potential of EVs to enter the BBB, they represent a complex system, making it difficult for drug delivery. Therefore, it is important to clarify their exact mechanism of action. The identification of miR-100a-5p and circZNRF1 is important for understanding their

mechanism of action in neurodegenerative disorders such as PD, offering novel treatments to overcome the challenges of drug delivery across the BBB.

Age-related macular disease (AMD)

AMD is the leading cause of irreversible blindness in elderly individuals worldwide [120]. In 2019, it was reported that more than 40 million people aged 40 and older were living with AMD in the United States [121]. This number is expected to rise with the increasing aging population [122, 123].

The pathogenic role of EVs in AMD In the early stages of AMD, drusen, which are yellowish lesions composed of accumulated macromolecules such as lipids, proteins, and cellular debris, can be found in one or both eyes [120] [124]. These lesions primarily appear between the basal lamina of the retinal pigment epithelium (RPE) and the inner layer of Bruch's membrane [124]. Medium- and large-sized drusen can lead to the degeneration of the RPE and photoreceptors, ultimately causing vision loss [122]. The major components of drusen include apolipoprotein E (ApoE), cholesterol, vitronectin (VTN), clusterin (CLU), complement components, and collagen [125].

The involvement of EVs in drusen formation was demonstrated by Wang et al., who found that the EV biomarker tetraspanin CD63 was presented in drusen [126]. Flores-Bellver et al. revealed that the protein associated with immune response, inflammation of RPE-derived EVs were increased under homeostatic conditions [127]. The EV proteome confirmed that the induced-primary RPE-derived EVs (secreted from both the apical and basal sides) contain proteins and lipids involved in drusen formation, including ApoE and ApoA2, and VTN, serum amyloid A-4 (SAA4), vimentin (VIM), amyloid-like protein 1 (APLP1), complement component 3 (C3), and alpha-crystallin B (CRYAB). Additionally, these proteins were enriched in key pathways of AMD, such as oxidative stress, immune response, and inflammation. Beyond drusen formation, ApoE and VTN have been demonstrated to induce inflammation within the retina by recruiting microglia and macrophages to the retinal area and secreting pro-inflammatory cytokines [128]. These findings suggest that pathogenic proteins could serve as targets for the therapeutic interventions.

EVs as biomarker for AMD diagnosis The traditional approach for AMD diagnosis is fluorescein angiography, in which the dye is injected into the retinal blood vessels to visualize chorioretinal circulation and detect retinal fluid leakage within seconds. This method provides information about active exudation of pathologic blood vessels into the retinal parenchyma. However, it is invasive and can have adverse side effects. An alternative method is optical coherence tomography (OCT), which non-invasively images the retinal area. However, OCT has limitations, particularly in distinguishing between dry and exudative late-stage AMD [120, 129].

Given that EVs reflect the condition of their donor cells, studying the protein profile of EVs secreted by AMD retinal and related cells presents a promising diagnostic approach. Several studies have demonstrated the potential of EVs as biomarkers for AMD. Tsai et al. conducted a proteomic assay on exosomes secreted by the aqueous humor of AMD patients [130]. LC–MS analysis identified 10 differentially expressed peptides, derived from five known proteins (Apolipoprotein 1, opticin, clusterin, complement C3, and prostaglandin) and one unknown protein. Two of these proteins, apolipoprotein 1 and C3, are components of drusen. Additionally, the study assessed the prognostic potential of EV proteins. It was observed that reduced expression of SERPINA1 and AZGP1 in EVs were observed after 12 weeks of anti-VEGF (ranibizumab) treatment [130]. Clusterin was also found to be significantly upregulated in EVs from high-risk AMD RPE cells, particularly from the apical side. Additionally, an increase in cytoskeletal and extracellular matrix proteins (e.g., vimentin, PACSIN1, fibronectin, reelin, and collagen), stress-related proteins (e.g., PPIA, Thy-1, HSP70, and S100-A8), and angiogenesis-linked proteins (e.g., brain acid-soluble protein 1, EDIL3, and aquaporin-1) were also noted. Gene ontology analysis revealed that these proteins are associated with oxidative stress, complement activation, immune response, amyloidosis, unfolded protein binding, and angiogenesis [130]. RPE cell natural polarisation is essential for their normal functions in photoreceptor maintenance (apical surface) and the interaction with the choroidal vasculature across Bruch's membrane (basal surface). Kurzawa-Akanbi et al. [131] focus on the composition of EVs secreted by different chambers of RPE cells and found that they are associated with high-risk AMD or low-risk AMD. EVs was collected from apical and basal chambers of low and high-risk RPE cells. The results of transcriptomic analysis noted that there were 1661 specific to apical EVs and 203 presents only in basal EVs. And the upregulated proteins in the apical and basal high-risk RPE EVs are known to regulate cytoskeletal function (MSN, KANK2, MTPN, SYNPO, TMSB10, ACTB, TMSB4Y). Apical high-risk RPE EVs were also enriched with transcripts encoding chaperone molecules (HSPB1, CDC37), but the basal high-risk RPE EVs highly expressed IGFN1 and CYP1B1. Above researches have preliminarily demonstrated the potential of complement components, inflammatory factors, angiogenesis-related proteins, and miRNAs in EVs for the diagnosis of AMD. We believed that future efforts

should integrate multi-omics technologies and clinical validation to further identify highly specific biomarkers and promote their translation into standardized diagnostic protocols.

EVs as therapeutic tools for AMD Currently, there is no known cure for AMD, particularly when the disease has progressed to the late stage. Nevertheless, a number of preventive measures have been implemented to avert vision loss during its early stages. While anti-VEGF therapy remains a highly effective treatment for age-related macular degeneration (AMD), it is imperative to explore the development of alternative therapeutic modalities that exhibit comparable efficacy while minimizing adverse effects for patients. [129, 132]. EVs secreted by the cells are recognized as effective therapeutic agents. It was observed that the administration of MSC-derived EVs into vitreous humor enhanced functional recovery of the retina in a rat model of retinal ischemia [133, 134]. This was accompanied by a reduction in neuroinflammation and apoptosis [134]. Furthermore, MSC-EVs have been observed to be internalized by retinal neurons and ganglion cells, as well as by microglia [134]. Besides, EV overexpressing miR424 significantly enhanced neuroprotection activities in retinal cells and reducing inflammatory cytokine production in retinal microglia, as well as attenuating oxygen free radicals in retinal Muller cells [133].

Mounting evidence is showing the association between MSC-EVs and various health outcomes. He et al. conducted a study on the effects of hucMSC-derived exosome (hucMSC-sEV) exposure to blue-light stimulated RPE cells and laser-induced retinal injury model [135]. Blue-light-stimulated RPE cells treated with hucMSC-sEV resulted in a reduction of VEGF mRNA expression over time and in a dose-dependent manner. The intravitreal injection of hucMSC-sEV has been employed in the laser-induced retinal injury treatment and was found to decrease of areas of CNV by attenuating fluid leakages [135]. Concurrent findings were reported that the intravascular injection of hucMSC-sEV attenuates subretinal fibrosis [136]. Their findings revealed that hucMSC-sEV exhibit elevated levels of miR-27b, which has been identified as a regulator of the epithelial-mesenchymal transition (EMT) process induced by transforming growth factor-beta 2 (TGF-β2) via inhibiting homeobox protein Hox-C6 (HOXC6) in ARPE19 cells. Additionally, the study revealed a suppression of RPE cell migration, which aligns with previous findings in glioblastoma [137] and glioma [138], where HOXC6 was identified as a crucial regulator of EMT through the TGF-β/SMAD signaling pathway. Human amniotic MSC-derived exosomes or conditional medium (hAMSC-sEV/CM) have also been found to exhibit therapeutic effects on H_2O_2-induced ARPE-19 cells [139] Treatment with hAMSC-sEV has been shown to alleviate oxidative damage and suppress ROS production by inhibiting the mitochondrial-mediated apoptosis pathway. Further analysis revealed that the PI3 K/Akt/FoxO3 axis plays a role in hAMSC-CM therapeutic effect. However, the specific type of cargo carried by the hAMSC-sEV/CM that regulates this signaling pathway remains unknown [140]. Another research shows that MSC-sEV reversed high glucose-induced senescence of ARPE-19 cells by enhancing antioxidant activity via suppressing PI3 K/AKT phosphorylation as well as nuclear factor erythroid 2-related factor 2 (Nrf2) expression along with its downstream target gene expression [141]. Treatment using embryonic stem cell-derived EV (ESC-EV) to the senescent RPE cells reduced SA-β-gal activity along with the level of mitochondrial membrane potential (MMP), ROS expression, and several SASP cytokines (IL6, IL1B, and TNFa). The underlying mechanisms of ESC-EVs therapeutic ability are through p38MAPK/p53 pathway inhibition via downregulating MKK3 protein expression in the cells [142]. The above-mentioned study revealed the protective effect of EVs and offered new insights for the EV-based treatment of AMD.

Skeletomotor system

Osteoarthritis

Osteoarthritis (OA) is a joint disease characterized by pain, swelling, and stiffness, along with limited movement [143], particularly affecting weight-bearing joint such as hands, knees, hips, neck, and lower back. It results from the breakdown of joint structures [144]. Historically, OA was believed to be the outcome of "wear and tear" damage; however, recent research has revealed more complex biological factors impacting the pathogenesis of OA in cartilage, bone, osteophyte, and ligaments [145, 146]. According to the data reported by WHO [143], 73% of the OA patients are over 55 years of age, with 60% of them being female, suggesting that females have a higher risk of suffering from this disease. Beside aging and sex, several factors, including genetic and epigenetic predisposition, obesity, endocrine influences, ethnicity, dysplasia, types of occupation, types of sports, and previous injury, also contribute to initiation, promotion and progression of OA [145]. Pro-inflammatory cytokines such as IL-1, IL-6, IL-7, IL-8, IL-17, IL-18, IL-20, IL-36, MCP-2, MIF, MIG, oncostatin M, bFGF, TGFα, S100 proteins, are found in OA joints, stimulating the production of matrix-degrading enzymes that lead to progressive destruction of the joints [147].

EVs as biomarker for OA diagnosis EVs secreted from OA synovial fluid have been shown to carry increased

levels of pro-inflammatory cytokines, which can inhibit chondrocytes proliferation [148]. Meanwhile, miRNA also play a role in the induction and diagnosis of OA. High-throughput sequencing revealed that 13 miRNAs were differentially expressed in EVs derived from the cartilage of OA patients, with 11 miRNAs downregulated, and 2 miRNAs upregulated. These miRNAs include miR-200a-3p (downregulated), miR-4454 and miR-443-3p (upregulated) [149]. Furthermore, in OA pathogenesis, miR-200a-3p regulate forkhead box protein C1 (FoxC1) by binding to its 3'UTR. The downregulation of miR-200a-3p results in the overexpression of FoxC1 and upregulation of fibronectin, MMP3, MMP13, and a disintegrin and metalloproteinase with thrombospondin motif 5 (ADAMTS-5) [150]. Notably, EVs from OA synovial fluid carry decreased levels of hyaluronic acid [151], a natural lubricant found in joint cartilage [151, 152]. Following this finding, it can be considered that the amount of hyaluronic acid contained in sEVs might act as a biomarker to aid in diagnosis and could be used to develop targeted treatment.

EVs as therapeutic tools for OA For years, traditional treatment of OA by direct injection of hyaluronic acid into the affected sites has been believed to cause local pain and swelling that lasts for days [153]. Currently, exosomal miRNAs dominate the field of OA treatment. Several studies have demonstrated that miRNA-containing EVs targeting chondrocyte yield positive results in OA treatment. EVs carrying miR-150-3p [154], miR-26a-5p [155], miR-204 [156], and miR-130b-3p [157] have been reported to target chondrocytes and successfully ameliorate OA through various mechanisms. In vitro studies show that miR-150-3p, carried by fibroblast-like synoviocytes-secreted EV (FLS-EV), targets T cell receptor-interacting molecule 14 (Trim14), NF-kB, and interferon-β, thereby reducing the production of pro-inflammatory cytokines. In vivo studies exhibit similar results when the articular cartilage of an OA model rat is treated with human FLS-EVs [154]. Proteomic analysis results revealed the potential for chondrocytes to regulate OA pain through cytokine secretion, including LDL Receptor Related Protein 1 (LRP1). In vivo observations show how miR-204 delivered through sEVss derived from MSC might play a role in reducing OA pain by blocking the interaction between neurons and cartilage via inhibition of the SP1-LRP1 signalling [156]. Synovial mesenchymal stem cells EV (SMSC-EV) may be well-suited for OA therapy, as cartilage and synovium originate from the same cell pool. It has been observed that miR-26a-5p carried by SMSC-EVs ameliorates OA through inhibiting the phosphatase and tensin homologue (PTEN), resulting in reduced levels of TNF-α and Bax, while the expression of IL-10 and Bcl-2 is elevated [155]. Similarly, miR-130b-3p carried in SMSC-EV promotes chondrocyte proliferation and migration, and suppresses chondrocyte apoptosis by downregulating LDL Receptor Related Protein 12 (LRP12), a protein that may activates P13 K/Akt-β catenin signalling pathway, which is known to induce apoptosis and inflammation of chondrocytes, thereby contributing to OA disease progression [157]. Furthermore, it was found that adipose-derived mesenchymal stem cells (ADSCs)-sEVs could suppress inflammation by downregulating TNF-α, resulting in enhanced chondrocyte proliferation and differentiation. Real-time quantitative PCR (RT-qPCR) results show elevated expression of miR-145 and miR-221 in synovial fluid treated by the sEVs [158]. Previous studies reported that treatment with miR-145 suppresses the expression of TNF-α, thereby decreasing inflammation, while a study reveals that miR-221 stimulates chondrocyte proliferation [159, 160].

In OA, mitochondrial dysfunction-induced accumulation of ROS leads to cartilage degradation and chondrocyte apoptosis, marked by elevated expression of MMP13 [161, 162]. Additionally, cartilage degradation resulting from mitochondrial dysfunction may also be caused by the increasing production of pro-inflammatory cytokines during aging, such as IL-1β and IL-18 [161]. Therefore, restoring mitochondria may represent potential therapy for OA. It is well established that sEVs and microvesicles can carry mitochondrial component, including mitochondrial DNA, RNA, and proteins [163]. A study conducted by Zheng et al.[164] revealed that primary chondrocyte-derived sEVs can ameliorate cartilage degradation by eliminating mitochondrial dysfunction. It has been reported that sEVs, especially those derived from MSCs, contain enzymes involved in the ATP-generating stage of glycolysis, such as glyceraldehyde 3-phosphatase dehydrogenase (GAPDH), enolase, and phosphoglycerate kinase (PGK) [165]. SEVS derived from primary chondrocytes may contain the similar enzymes, as the ATP levels in chondrocytes were found to increase after treatment, implying restored mitochondrial function. Additionally, following sEVs treatment, M2 macrophage infiltration increased while M1 macrophages decreased in cartilage, indicating the immunomodulating capability of EVs [164] A study by Yu et al. [166] obtained similar results, but found that microvesicles exhibit more significant mitochondrial restorative effects than sEVs in rescuing mitochondrial dysfunction. Microvesicles derived from bone marrow MSC (BMSCs) may have the ability to directly transfer healthy mitochondria to impaired chondrocytes. Both types of EVs exhibit positive results in alleviating OA pathogenesis. Through endocytosis, they successfully revive mitochondrial function, downregulate MMP13 expression, and upregulate the expression of

type II collagen and aggrecan [164, 166]. However, it is noteworthy that not all cell-derived mitochondria exhibit the same benefits. Skeletal muscle and MSC-derived mitochondria are the most studied and have been proven to yield the best results [167, 168]. This suggests that the source of EVs may be an important part in determining their therapeutic efficacy in OA treatment.

Osteoporosis

Osteoporosis is a common age-related bone disease characterized by bone mass loss and reduced bone mineral density, which increases the risk of fractures [169]. The epidemiology of this disease may vary in sex and race, but most of the cases occur in menopausal women due to estrogen depletion, which leads to higher urinary calcium excretion and disrupts bone remodeling, consequently lowering bone mineral density [169–171]. Nutritional deficiencies, such as vitamin D insufficiency, and sedentary lifestyles also escalate the risk of aged-related osteoporosis [172]. Since early-stage of osteoporosis is most-likely asymptomatic, it is often only diagnosed after a fracture occurs, especially in the hip, vertebrae, and forearm regions [170].

EVs as biomarker for osteoporosis diagnosis In osteoporotic plasma, EV secretion is elevated, with higher expression of miR-335-5p, miR-320a, miR-483-5p, and miR-21-5p, as determined by real-time PCR. Additionally, EVs derived from osteoporosis patient plasma exhibited lower levels of Wnt molecules, including Wnt1, Wnt5a, Wnt7a, and Wnt9a, while no significant changes were observed in Wnt5b mRNA expression [173]. This suggests that the Wnt signaling pathway may play a role in regulating osteoporosis. The Wnt signaling pathway is known to balance bone remodeling by promoting bone formation and reducing bone resorption [174]. MiR-335-5p has been found to positively regulate the Wnt signaling pathway, thereby promoting osteogenesis [175]. Conversely, upregulated miR-320a inhibits Wnt signaling, contributing to osteoporosis progression [176, 177]. The Wnt signaling pathway is crucial for bone formation, and it represents a potential target for osteoporosis therapy. Similarly, the upregulation of miR-483-5p is involved in osteoporosis pathogenesis through the activation of the PI3 K/AKT pathway, a pathway involved in cell proliferation, growth, and migration [178, 179]. Given these findings, the PI3 K/AKT pathway emerges as a promising target for osteoporosis treatment. However, since P13 K/AKT pathway has various roles, including the regulation of osteoblast and osteoclast function, further studies are required to explore treatment targeting P13 K/AKT pathway [180, 181]. As the P13 K/AKT pathway can be regulated by miRNAs, understanding their interplay could lead to the development of novel treatment of osteoporosis.

Recent studies have also observed that, compared with control and osteopenic patients, osteoporotic patients have a significantly increased number of EVs, as well as higher protein content. Proteomic analysis revealed that osteopenic EVs lack of fibrinogen, vitronectin, and clusterin expression, but are abundant in the coagulation factors and apolipoproteins, a pattern that also observed in osteoporotic EVs. The receptor activator of NF-κB ligand (RANKL) is an important regulator of bone metabolism and remodelling. Flow cytometry results show that $RANKL^+$ EVs are highly increased in osteoporotic plasma and positively associated with serum RANKL levels but negatively related to T-score (for classification of osteopenia and osteoporosis) at the lumbar spine and femoral neck. In vitro studies demonstrated that OP EVs inhibit MSCs' differentiation into osteoblasts while promoting the expression of OSTERIX and RANKL in mature osteoblasts. Additionally, OP EVs were found to support osteoclastogenesis of healthy donor peripheral blood mononuclear cells (PBMCs), similar to the effects of RANKL and macrophage-colony stimulating factors (M-CSF) treatments. After miRNome analysis, it was found that miR-1246 is the most highly upregulated miRNA in osteoporosis-EVs, and treatment of healthy osteoclast precursors with the miR-1246 mimic enhanced the osteoclasts differentiation. The differentially expressed miRNAs were found to be involved in the regulation of vitamin D receptor signalling and osteoclast differentiation pathways, highlighting the correlation between EVs and the pathogenesis of osteoporosis [182].

Nano-liquid chromatography tandem mass spectrometry (nLC–MS/MS) analysis revealed that certain proteins are highly expressed in EVs from various osteoporosis stage. Von Willebrand Factor (VWF), Albumin (ALB), Coagulation factor V (FA5), and Proteoglycan 4 (PRG4) are highly expressed in healthy control (CN) EVs, while Angiotensinogen (ANGT), Adiponectin, c1q, and collagen domain containing (ADIPOQ), Serum Amyloid A1 (SAA1), and Immunoglobulin Heavy Variable 3–72 (IGHV3-72) are highly expressed in OPN EVs. EVs from OP patients show high expression of TETN, TFS, SAA1, and V2-17 [182].

EVs as therapeutic tools for osteoporosis Another significant protein carried by EVs is Tropomyosin-1. Treatment of osteoporosis using EVs derived from young osteocyte carrying this protein, helps balance bone formation and bone resorption [183]. Tropomyosin-1 is a protein regulator for cell proliferation and activates YAP1 gene, thus promoting osteogenic [184].

Proliferating cell nuclear antigen (PCNA), carried by sEVs, has shown potential in mitigating bone loss associated with osteoporosis. PCNA is known to participate in bone formation by regulating β-actin-cytoskeleton remodelling. During RANKL-induced osteoclast differentiation, PCNA translocate from nucleus to the cytoplasm, and accumulates in the actin belt of mature osteoclasts [185]. PCNA were found to be abundant in UCMSC-derived EVs. Treatment with these EVs in aged BMSC significantly improved osteogenic differentiation, as marked by increased expression alkaline phosphatase (ALP), RUNX Family Transcription Factor 2 (RUNX2), Bone Morphogenetic Protein 2 (BMP2), and osteocalcin (OCN). Concurrently, it inhibited adipogenic differentiation, as indicated by reduced expression of fatty acid binding protein 4 (FABP-4), peroxisome proliferator-activated receptors γ (PPAR-γ), and lipoprotein lipase (LPL). In vivo studies have revealed that UCMSC-derived EVs can enhance the bone surface to tissue volume ratio (BS/TV) and trabecular number. Microcomputed tomography (μCT) and H&E staining revealed an increase in osteoblast number and osteocalcin expression, respectively [186]. These findings suggest that PCNA-carrying sEVs holding therapeutic potential for osteoporosis.

Systemic disorders

Immunosenescence

Aging leads to gradual decline in immune system function, a process known as immunosenescence. Immunosenescence is characterized by changes in lymphoid organs and increased susceptibility to infections, autoimmune diseases, and malignancies [187]. This decline is characterized by a reduced number of B cell, B cell repertoire, naïve T cell, and regulatory T cell and alterations in hematopoietic differentiation. Memory T cells and effector T cells are increased in aged mice comparing to young mice [188, 189]. Immunosenescence involves complex biological changes in immune cells, including their secretory phenotype, circulatory status, metabolic state, and epigenetic status [187, 190]. Targeting immunosenescent phenotypes may offer novel therapeutic opportunities for diseases like cancer and autoimmune disorders.

The pathogenic role of EVs in immunosenescence Like other senescent cells, senescent immune cells secrete increased amount of SASP, p16 and p21, contributing to age-related inflammation termed as inflammaging [191, 192]. Aging macrophages play a crucial role in immunosenescence [193]. Macrophages from aged bone marrow (ABMM) can propagate SASP proteins to multiple tissues via their secreted EVs [194]. miRNA enrichment analysis revealed that miR-378a-3p is enriched in EVs from ABMM, while miR-191-5p is more abundant in EVs from young bone marrow-derived macrophages (YBMM) [194]. Bioinformatics analysis identified that both miRNAs target peroxisome proliferator-activated receptor α (PPARα), a nuclear transcription factor that regulates key genes involved cellular senescence and organ dysfunction [195, 196]. Mandibuloacral dysplasia-(MAD) individuals with homozygous laminA/C p.R527 C mutations exhibit overlapping progeroid symptoms [197], as well as the hyperactive immune response accompanied by higher expressions of IL-6, IL-9, and IL-10 in the serum and increased terminally-differentiated senescent T cell [198]. The author believed that MAD iPSC-derived Mesenchymal stem cells (MAD-iMSC) extracellular vesicles can faithfully recapitulate both the clinical manifestation and pathophysiology of complex multi-trait disorders [199]. They found that MAD-iMSC-EV caused defective mitochondrial homeostasis and enhanced collagen deposition, as well as worsened the fibrotic score compared with the vehicle control. The upregulated IL-6 expression in EVs was thought to be the reason for these disturbances [198].

EVs as biomarker for immunosenescence Recent studies have demonstrated differences between EVs isolated from young and aged mice with fractures. In young mice, fractures lead to elevated release of sEVs and microvesicle from immune cells, such as NK1.1, CD63, CD4, and CD9 positive cells, which are believed to aid in the healing process [189]. For example, B cell-derived sEVs from fracture calluses have been shown to promote fracture healing by inhibiting osteoblast differentiation and inducing osteoclast formation [200]. However, the specific compounds within EVs responsible for their therapeutic effects remain unidentified. Additionally, it has been observed that aged mice have a higher number of apoptotic cells and tend to secrete excessive number of large EVs (LEVs), which carry significant amounts of pro-inflammatory cytokines, including IL-13, IL-4, IL-10, IL-1β, IL-6, IFN-γ, IL-17 A, and TNF-α. These pro-inflammatory LEVs potentially hinder the healing process and contribute to inflammaging [189]. Tsukamoto et al. revealed that EVs secreted in the serum of aged mice carry overexpressed miRNA-192, an aging-associated immune regulatory microRNA [201]. Mitochondrial homeostasis is crucial for immune cell function. Zhang et al. demonstrated that the respiring mitochondria cargo in EVs of several types of immune cells are gradually decreases with age [202]. And the number of plasma EV derived from $CD31^{+}$HSCs, B cells, T cells, natural killer (NK) cells and $CD9^{+}CD31^{+}$HLA-ABC^{+}HLA-$DRDPDQ^{+}$ antigen presenting cells also show the age-related decline pattern [202]. And depends on the size of the EVs, they found that the smaller EV subtypes (small EV and microvesicle) account for the vast majority of the age-associated

EV subpopulations. An interesting hypothesis is that this decline may be due to higher demand for parent cells or increased tissue uptake, indicating a direction for follow-up research on the present topic.

EVs as therapeutic tools for immunosenescence During aging, hematopoietic stem cells (HSCs) tend to shift toward a myeloid-biased differentiation, leading to increased myelopoiesis and reduced lymphopoiesis [158]. This imbalance contributes to elevated production of pro-inflammatory cytokines, such as TGF-β and IL-10. Recent studies suggest that EVs secreted by HSCs play a crucial role in regulating HSC fate. The mitochondrial fatty acid oxidation (FAO)-NADPH-cholesterol axis drives EV biogenesis, leading to increased EV secretion from HSCs. Cholesterol plays a key role in facilitating the tetraspanin cycle, which regulates EV biogenesis and cargo selection, ensuring proper loading of CD9 and CD63 into HSC-derived EVs. Notably, stromal cells preferentially take up EVs secreted by HSCs over those from other progenitor cells. Treatment with these EVs stimulates stem cell factor (SCF) expression in both stromal cells and HSCs, fostering a microenvironment that supports HSC maintenance [159]. These findings highlight the potential of EVs in mitigating immunosenescence by modulating HSC fate and preserving hematopoietic homeostasis. The aging-associated immune regulatory miR-192 also alleviate hyperinflammation and immune dysfunction by internalization [203]. In the context of inflammation, miR-192 has been shown to inhibit M1 macrophage polarization [204]. Additionally, studies indicate that miR-192 can suppress cancer cell proliferation, migration, and invasion, thereby limiting tumor progression [205]. Tsukamoto's findings further underscore the therapeutic potential of EVs derived from senescent cells, particularly in mitigating inflammatory responses and restraining aberrant cell proliferation [201]. As immunosenescence is associated with the decline of immune functions, we revisit this issue in the section below.

Cancer

Nowadays, cancer has been categorized as an age-related disease. In 2020, 64% of the latest cancer cases were in the 60 and older age group [206]. The cause of tumorigenesis can be divided into internal factors (inflammation, epigenetic alterations, DNA damage, mitochondrial dysfunction) and external factors (lifestyle, environment, exposure to toxic chemical substances). These factors will accumulate in the body and consequently appear in old age, therefore aging becomes the primary risk of cancer [207].

The pathogenic role of EVs in cancer The effects of aging lead to the accumulation of senescent cells, which are in a permanent state of cell cycle arrest induced by cellular stress [208]. These cells secrete a group of molecules known as the SASP, facilitating the paracrine effects of senescent cells, contributing to a pro-tumorigenic environment [209]. For example, in oxidative stress-induced senescent cells excessively secrete sEVs, which enhancing the proliferation of MCF-7 breast cancer cells. Immunoblotting has revealed increased expression of EphA2 in sEVs. Mechanistically, ROS induces EphA2 phosphorylation and promoting EphA2 endocytic internalization during sEV biogenesis [210]. EphA2 interacts with its ligand ephrin-A1 [211], activating the Erk pathway for cell proliferation [210]. Senescent lung fibroblasts also contribute to lung cancer progression via EV secretion. Exosomes from these cells carry MMP1, which activates Protease-activated receptors1 (PAR1) through the PI3 K/AKT/mTOR pathway, a signaling cascade that promotes cell survival, growth, and cycle progression [179, 212]. Consequently, these EVs enhance the proliferation of non-small cell lung cancer (NSCLC) cells [212]. Additionally, MMP1 contributes to resistance against erlotinib, a chemotherapy drug, thereby reducing NSCLC treatment efficacy [213]. These findings highlight MMP1 as a potential therapeutic target for NSCLC. Senescent fibroblasts have also been implicated in melanoma progression by promoting angiogenesis [214]. The senescent fibroblast-derived EV reduced the expression of the tetraspanin CD9, which leads to angiopoietin-like protein 2 (ANGPTL2) overexpression. The impact of these EVs on melanoma occurs indirectly through endothelial cells. ANGPTL2-enriched EV induces sprouting in endothelial cells and angiogenesis in melanoma [215].

Tumor cells can also secrete EVs that contribute to tumor growth and metastasis. In ovarian cancer, exosomes isolated from patient plasma exhibit upregulated expression of SOX2-OT [216], a long non-coding RNA known to regulate cancer cell proliferation [217]. Bioinformatic analysis suggests that the exosomal SOX2-OT serves as a miR-181b-5p sponge and promotes stearoyl-CoA desaturase 1 (SCD1) [216], which is highly expressed in ovarian cancer cells, plays a crucial role in facilitating malignancies and protecting cells from ferroptosis-induced death [218]. Additionally, tumor cell-derived EVs have been identified as key contributors to immunosenescence, thereby promoting tumor progression and therapeutic resistance. Carcinoma tissues secrete EVs encapsulate circCCAR1, a circular RNA which could subsequently internalized by $CD8^+$T cells [219]. This uptake facilitates $CD8^+$T cell dysfunction by stabilizing the PD-1 protein through PD-1 deubiquitination, thereby impairing the effector immune response.

Consequently, $CD8^+$ T cells lose their cytotoxic function and exhibit reduced secretion of TNF-α, IFN-γ, and granzyme B, which are critical for tumor cell eradication [219]. These findings highlight the multifaceted role of EV-mediated molecular signaling in immune evasion and therapeutic resistance in cancer.

EVs as biomarker for cancer diagnosis Cancer treatment is often a race against time, making accurate and non-invasive diagnostic strategies crucial for early detection. Among various liquid biopsy approaches, studying EVs secreted by cancer cells has gained significant attention. The functional biomarkers in EV roughly contain miRNA and protein.

Several studies highlight EVs as promising diagnostic and prognostic tools. Nobrega et al. investigated EV protein profiling in prostate cancer (PCA) [220]. They collected EV samples from 30 prostate cancer patients, 20 with localized PCA and 10 with metastatic PCA. RT-qPCR analysis revealed three miRNAs, miR-21-5p, miR-375 (specificity of 93.93%), and miR-1290-3p (specificity of 85.7%), that were highly expressed in localized PCA EVs compared to controls. In metastatic PCA showed increased expression compared to controls, including miR-21-5p (specificity of 100%), miR-200c (specificity of 100%), miR-1290-3p (specificity of 85.7%), and miR-375 (specificity of 93.93%). These findings suggest that EV miRNA profiling can facilitate prostate cancer detection and differentiation between disease stages. Further, Nakamura et al. focused on miRNAs expressed in EVs from early-stage pancreatic cancer samples [221]. Through RT-qPCR and bioinformatic analysis, they identified eight differentially expressed miRNAs in EVs from 44 stage I/II pancreatic cancer patients compared to 57 non-diseased controls: miR-145-5p, miR-200b-3p, miR-429, miR-1260b, miR-145-3p, miR-216b-5p, miR-200a-3p, and miR-217-5p.

For breast cancer diagnosis, Xu et al. conducted proteomic profiling of EVs secreted in the serum of 126 breast cancer (BC) patients and 70 healthy donors [222]. Using LC–MS and bioinformatic analysis, they identified 485 unique proteins in BC-derived EVs and found that these proteins were enriched in immune response, metabolism, and metastasis pathways. The XGBoost classifier identified seven key proteins unique in BC-derived EVs: IGHV3-23, MMP9, AHNAK, phosphoribosylaminoimidazole succinocarboxamide synthetase (PAICS), Von Willebrand Factor (VWF), Angiopoietin-like 6 (ANGPTL6), and Proteasome Activator Subunit 1 (PSME1) [222]. The team also analyzed EV proteomic landscapes for different BC subtypes: Luminal A-EVs were enriched in proteins related to proteolysis, protein folding, necroptotic cell death regulation, cellular stress responses, and external stimuli responses. Luminal B-EVs predominantly expressed proteins involved in tight junctions, ECM-receptor interactions, fructose/mannose metabolism, glucose metabolism, and insulin signaling pathways. HER2-enriched-EVs contained proteins associated with hydrogen peroxide responses, ErbB2/ErbB3 signaling, keratinization, tyrosine metabolism, and arginine/proline metabolism. Triple-negative breast cancer (TNBC) -EVs exhibited proteins linked to platelet activation, antigen processing and presentation, actin cytoskeleton regulation, angiogenesis, and cell motility [222]. The study also identified Transaldolase 1 (TALDO1) as a unique biomarker in EVs from distant BC metastases. Fecal-derived EVs present a promising avenue for colorectal cancer detection. Zhang et al. analyzed these EVs to develop non-invasive diagnostic and prognostic markers for colorectal cancer[223]. Their protein-based screening combined with Western blotting identified CD147 as the colorectal cancer-specific proteins in fecal EVs: CD147 and cell surface A33 antigen (A33). Both proteins reside on the EV surface, with CD147 previously recognized for its role in promoting cancer malignancy [224], and A33 expressed in 95% of colorectal cancer cases [225]. By investigated the proteomic profile of extracellular vesicles and particles (EVPs) from 426 human tissue explant samples, 862 EVP unique proteins were identified as pan-EVP markers [226]. Interestingly, periostin, S100 A13 and other 50 proteins are pancreatic cancer specific EVP proteins, but HIV-1 Tat interactive protein 2 (HTATIP2) and methyltransferase like 1 (METTL1) are the only two lung cancer specific EVP proteins [226]. This imbalance implied EVP generation and cargo encapsulation is dynamically regulated by tumor-specific biology.

EVs as therapeutic tools for cancer Numerous studies continue to explore the therapeutic potential of EVs in cancers, aiming to uncover their underlying mechanisms, including key effector molecules and signaling pathways involved. EVs secreted by immune cells are usually as effective as the donor cells. EVs secreted directly by $CD45RO^-CD8^+$T exhibit direct antitumor effects. In oestrogen-driven endometrial cancer (UCEC), several miRNAs are downregulated during the UCEC cell proliferation and invasion. Notably, miR-765, one of the most significantly downregulated miRNAs, is co-localization with $CD8^+$T cells. $CD45RO^-CD8^+$T T cell-derived exosomes release more miR-765 than that from cells. MiR-765 negatively regulates PLP2 expression of UCEC, then suppresses cancer cell growth and epithelial-mesenchymal transition (EMT). Since PLP2 is known to promote proliferation, EMT, invasion, and metastasis in ovarian cancer, these findings underscore the potential of $CD8^+$T cell-derived EVs in anticancer therapy [227]. IL-2

is widely known for directly activating $CD8^+$T cell [228]. Interestingly, IL-2 can also indirectly enhance $CD8^+$T cell activation via EVs secreted by $CD4^+$T cells. In a melanoma mouse model, IL-2-induced $CD4^+$T cell-derived EVs significantly enhanced $CD8^+$T cell activation, leading to tumor killing. Further analysis identified three miRNAs: miR-155-5p, miR-215-5p and miR-375, as key mediators of this effect [229]. While the precise mechanisms remain unclear, previous research suggests that miR-155 targets inositol 5-phosphatase SHIP1, a negative regulator of the PI3 K/AKT pathway, as well as several inhibitors of the JAK/STAT5 pathway, such as SOCS1 and Ptpn2. These interactions contribute to $CD8^+$T cell activation [230]. Given that the PI3 K/AKT and JAK/STAT5 pathways regulate $CD8^+$T cell expansion and survival, especially in response to cytokines like IL-2, IL-7, and IL-15 [231], further research should focus on elucidating the role of $CD4^+$T cell-derived EVs in promoting $CD8^+$T cell function through these signaling pathways.

Chimeric antigen receptor T (CAR-T) cells, which are genetically engineered T cells expressing a tumor-targeting receptor, represent a breakthrough in cancer immunotherapy. Interestingly, CAR-T cell-derived EVs also express CAR on their surface, enabling them to retain tumor-targeting properties while exhibiting lower cytotoxicity compared to direct CAR-T cell treatment. One advantage of EV-based therapy is that CAR-T cell-derived EVs lack programmed cell death protein 1 (PD1), making them resistant to PD-L1-mediated immunosuppression [232]. The therapeutic potential of CAR-T cell-derived EVs has been demonstrated in various cancers. In breast cancer, EVs isolated from mesothelin-targeted CAR-T cells effectively inhibited the growth of MSLN-positive TNBC cells by delivering perforin and granzyme B [233]. However, further studies are required to fully elucidate the underlying therapeutic mechanisms in breast cancer therapy. In leukemia therapy, EVs from CD19-targeted CAR-T cells carry CD19 CAR protein on their surface, allowing for highly specific targeting of CD19-positive leukemia B cells. Treatment with these EVs successfully induced pro-apoptotic gene expression in CD19-positive cells, while no toxicity was observed in CD19-negative cells. Notably, while other cell types (e.g., HL60 and K562) internalized these EVs, no cytotoxic effects were observed, highlighting the specificity of EV-based therapy [234]. Despite these promising findings, further studies are needed to fully elucidate the molecular mechanisms underlying CAR-T cell-derived EV therapy.

Let-7b-5p has also been shown to negatively regulate cancer cell growth in both breast and colon cancer cell [235, 236], suggesting its role as a key effector molecule in EV therapy. let-7b-5p is enriched in NK-EVs and successfully suppressed pancreatic cancer cell proliferation [237, 238]. Bioinformatics analysis using miRWalk identified several let-7b-5p target genes, including CDK6, CCNA2, and AURKB etc., all of which are implicated in cancer progression. Among these, CDK6 was significantly downregulated following miRNA treatment [238]. Given this, the study hypothesized that NK-EVs inhibit pancreatic cancer progression via the let-7b-5p/CDK6 axis [238]. EV-based immunotherapy represents a promising approach in cancer treatment due to its ability to modulate the tumor microenvironment, enhance immune cell function, and selectively target malignant cells. While the field is still evolving, continued research is essential to fully harness the therapeutic potential of immune cell-derived EVs in cancer immunotherapy.

EVs also can serve as drug delivery platforms in cancer therapy. Biological modifying EVs or encapsulating small-molecule compounds in EVs are two well established ways for greatly amplifying the advantages of the EV application. Li et al. provide a systematic review of the drug delivery of EV-based system in tumor treatment [239].

Metabolic disorders

Metabolic alterations can arise due to various factors, including aging, obesity, lifestyle choices, genetics, and environmental influences. Among these, aging is recognized as the most significant risk factor for metabolic dysfunction [240]. Age-related metabolic alterations are driven by factors such as chronic inflammation, oxidative stress, and mitochondrial dysfunction. These changes can impair β cell function, disrupting insulin secretion and contributing to metabolic dysregulation [241].

The pathogenic role of EVs in metabolic disorder As mentioned before, pro-inflammatory conditions have been shown to drive metabolic alterations in cells, often leading to macrophage polarization toward the M1 phenotype, which is characterized by the secretion of pro-inflammatory cytokines. A growing body of research suggests that EVs secreted by M1 contribute to insulin resistance. Qian et al. found that M1-derived EVs carry miR-212-5p, which targets SIRT2, a key regulator of insulin secretion within the AKT/GSK-3B signaling pathway [242, 243]. Similarly, Li et al. identified miR-143-5p in M1-derived EVs, which targets the kinase phosphatase-5, a crucial modulator of obesity-induced insulin responses that prevents the onset of insulin resistance and metabolic abnormalities [244, 245]. Both studies demonstrated that suppression of these targets results in the inhibition of the AKT/GSK-3B signaling pathway, ultimately leading to reduced insulin secretion and the development of insulin resistance, a hallmark of diabetes.

Beyond macrophages, adipose tissue-derived EVs and adipose-derived stem cell (ADSC)-derived EVs also contribute to insulin resistance. Notably, adipocytes cultured under insulin-resistant conditions secrete EVs enriched with sonic hedgehog (Shh) proetin, which promotes M1 macrophage polarization via activation of the Ptch/PI3 K pathway [246]. Additionally, adipocyte-derived EVs contain miR-27a, a key molecule linked to insulin resistance [247]. MiR-27a exerts its pathogenic effects by targeting PPARγ, which is essential for adipocyte differentiation and proliferation [247, 248]. In conditions of obesity and insulin resistance, PPARγ expression is downregulated in adipose tissue, but the precise mechanisms linking its inhibition to insulin resistance remain to be fully elucidated.

Another hallmark of metabolic disorders is β-cell dysfunction, particularly under diabetogenic and pro-inflammatory conditions. Dysfunctional β cells secrete small extracellular vesicles (sEVs) that overexpress CXCL10, a chemokine localized on the surface of these EVs. CXCL10 binds to its receptor CXCR3 on neighboring β cells, activating the NF-κB and JAK/STAT1 signaling pathways, thereby amplifying inflammatory responses [249]. Previous studies have demonstrated that STAT1 and NF-κB signaling pathways work synergistically to induce ER stress in pancreatic β cells, partly through the inhibition of the ER Ca^{2+} pump SERCA2b, leading to ER Ca^{2+} depletion. Additionally, NF-κB-mediated nitric oxide (NO) production exacerbates this stress [249]. In parallel, another study found that EVs secreted by dysfunctional β cells under diabetic conditions further contribute to a pro-inflammatory microenvironment by inducing IL-6 and IL-1β expression in vitro. These EVs carry 43 differentially expressed miRNAs, including miR-375, which has been shown to promote IL-1β, IL-6, and TNF-α production in both acinar cells and macrophages [250]. These findings suggest that miRNA-enriched EVs from dysfunctional β cells amplify inflammatory cascades and contribute to both metabolic and immune dysregulation. Another pathogenic mechanism of miR-27a in EVs involves its targeting of the L-type Ca^{2+} channel subtype CaV1.2, which plays a crucial role in insulin secretion by pancreatic β cells [251]. As a voltage-gated calcium channel, CaV1.2 regulates insulin release, and its repression or mutation leads to impaired β-cell function and the onset of insulin resistance [251, 252].

Notably, insulin resistance can also be triggered by circadian rhythm disruption, a condition that may be reflected in EV cargo from adipocytes. Upregulation of miR-22-3p has been reported in adipocyte-derived EVs under this condition [253]. Previously, miR-22 was found to be highly expressed in the liver and was upregulated in insulin-resistant states. This miRNA targets the Wnt-responsive transcription factor Tcf7, contributing to impaired gluconeogenesis and elevated circulating glucose levels in both mouse models and human cells [254].

These findings suggest that miRNA-enriched EVs serve as key mediators in the pathogenesis of insulin resistance, further linking metabolic dysfunction to immune dysregulation.

EVs as biomarker for metabolic disorder The protein profile of EVs reflects the physiological state of the parent cell, making EVs valuable indicators of metabolic changes. This principle was observed in adipose tissue-specific microRNA (miRNA)-processing enzyme Dicer knockout (AdicerKO) mice, which exhibit an enlargement of brown adipose tissue (BAT) and a reduction in white adipose tissue (WAT). Proteomic analysis of small EVs (sEVs) isolated from the serum of AdicerKO mice identified 349 proteins, of which 86 were differentially expressed compared to control EVs, 40 were upregulated, while 46 were downregulated [255]. Gene Ontology Enrichment and Pathway Analysis revealed that the downregulated proteins were primarily involved in retinol metabolism and complement activation, including significant reductions in retinol-binding protein 4 (RBP4), transthyretin (TTR), complement factor H (CFH), and complement components C3, C5, C7, and C9. Conversely, downregulated proteins were also implicated in inflammatory responses and iron metabolism, with notable decreases in serum amyloid P-component (APCS), serum amyloid A protein (SAA1), C1 esterase inhibitor (Serping1), ceruloplasmin (CP), haptoglobin (HP), hemopexin (HPX), and extracellular superoxide dismutase [Cu–Zn] (SOD3) [255]. Additionally, adiponectin, a key regulator of insulin sensitivity, cell survival, and inflammation, was significantly downregulated in serum sEVs from AdicerKO mice. Given its role in metabolic regulation, the decreased presence of adiponectin in sEVs suggests phenotypic changes consistent with insulin resistance and chronic inflammation [255, 256]. Beyond ADIPOQ, perilipin A expression in adipocyte-derived EVs serves as another indicator of metabolic alterations. Perilipin A, a lipid droplet-associated protein, is upregulated in EVs from adipocytes in obesity models [257, 258]. However, its direct link to insulin resistance remains unclear [258].

In addition to adipocyte-derived EVs, urinary EVs offer another promising source for detecting metabolic changes. EVs secreted by mesangial cells in the urine of diabetic mouse models show upregulation of miR-130a and miR-145, along with downregulation of miR-155 and miR-424 [259]. Similarly, urine-derived exosomes from diabetic patients exhibit upregulation of miR-133b, miR-342, and miR-30a, and all of these are known to induce insulin resistance [260]. Specifically,

miR-133b overexpression impairs mitochondrial respiration, glucose uptake, glycolysis, ATP production, and cellular energy balance by targeting Kruppel-like factor 15 (KLF15) [261]. Meanwhile, miR-342 targets regulatory factor X3 (Rfx3), leading to insulin resistance and impaired gluconeogenesis [262].

Taken together, these findings demonstrate that insulin resistance and metabolic dysfunction are mirrored in the proteomic and miRNA profiles of EVs. As such, EV-based biomarkers hold significant potential for diagnosing metabolic disorders and identifying molecular targets for therapeutic intervention.

EVs as therapeutic tools for metabolic disorder ADSCs play a dual role in both disease progression and potential therapeutic intervention of metabolic disorders. ADSCs exert a paracrine effect through the secretion of EVs, which have been observed to improve insulin resistance by modulating immune cell function. These ADSC-EVs carry signal transducer and activator of transcription 3 (STAT3) [263], regulating macrophage polarization through JAK/STAT, SHH, PI3 K/AKT, Wnt, MAPK, and NF-κB signaling pathways, [264]. Additionally, STAT3 promotes the expression of arginase 1, an enzyme that further reinforces M2 polarization by facilitating the production of ornithine, a metabolite associated with anti-inflammatory effects [265]. By EV induced ADSC-macrophage cross talk, the inflammation reduction, and beiging in white adipose tissue (WAT) of diet-induced obese mice could be observed.

Beyond ADSC-derived EVs, M2 macrophages themselves can secrete exosomes that enhance insulin sensitivity and glucose tolerance. Adipose tissue macrophage has reported benefit in causing insulin sensitization, but EVs harvested from M2-like ATMs in lean mouse eWAT is very sparse [266]. Ying et al. used IL4/IL13-induced M2-like bone marrow-derived macrophages (M2-BMDM) as the parent cell. The M2-BMDM-EVs could be detected in liver, skeletal muscle, and adipose tissue after intravenous injection [267]. These EVs enriched in miR-690, which is subsequently delivered to adipose tissue. Overexpression of miR-690 has been shown to upregulate pathways such as JAK/STAT and insulin signaling while concurrently suppressing glutathione metabolism. Mechanistically, miR-690 targets Nadk (encoding NAD^+ kinase) and inhibites nicotinamide adenine dinucleotide (NAD^+) converting into $NADP^+$ [267]. Inhibition of Nadk has been linked to increased insulin sensitivity.

These findings underscore the potential of ADSC-derived and M2 macrophage-derived EVs as therapeutic agents for mitigating insulin resistance and metabolic dysfunction by reshaping the inflammatory and metabolic landscape of adipose tissue.

Challenges and future prospects of extracellular vesicles as delivery vehicles in age-related diseases

EVs have emerged as potential delivery vehicles for disease treatment, supported by numerous animal experiments demonstrating their advantages in treating various diseases. In recent years, EVs have also been tested in clinical trials for age-related diseases (Table 1). Most EVs used in therapeutic applications are predominantly derived from MSCs (BMSC, ADSC, UCMSC) and dendritic cells. Nevertheless, EV-based therapies are still in the early stages of development, and multiple strategies are needed to overcome current challenges in order to enhance the efficiency and efficacy of these treatments.

Challenges in clinical translation

Numerous studies have demonstrated the therapeutic potential of extracellular vesicles (EVs), with several EV-based treatments progressing into preclinical and clinical trials (Table 1). However, we also acknowledge that multiple challenges have hindered the advancement of EV-based therapies, particularly at the clinical trial stage. The factors contributing to this translational gap remain incompletely understood.

One major bottleneck lies in the limitations of current EV production and isolation methodologies. These approaches often yield insufficient quantities of EVs, posing significant challenges for downstream applications that require large-scale production. Furthermore, the absence of standardized protocols contributes to inconsistencies in EV quality and composition across different batches and studies, thereby limiting reproducibility and therapeutic reliability [268, 269]. Contamination during EV production, such as with viruses, bacteria, or fungi, also presents a critical safety concern. Rigorous quality control measures are thus essential to ensure the sterility and safety of EV products prior to clinical administration [270].

Another challenge is the inherent variability in EV content and composition, which can result in unpredictable therapeutic outcomes. This variability complicates the determination of effective dosages and treatment protocols and also makes it challenging to elucidate the mechanisms underlying EV-based therapies [271]. Factors such as the cell type of origin, cellular physiological state, environmental stressors, and the specific disease context can all influence EV content, affecting their downstream signaling properties and biological efficacy in recipient cells [272, 273]. Beyond general markers for biological activity (e.g. CD9, CD63, CD81, etc.) for EV-based

Table 1 Clinical trials based on EV therapy

No.	Conditions	CT ID	Title	Study start	Study completion	Feature	Donor cell	Phase
1	Skin Aging	NCT06567119	Preliminary Evaluation of the Safety and Tolerability of SPOT-mRNA01 Subcutaneously Administered in Healthy Subjects	01–10–2024	15-12-2025	SPOT-Mrna01 (COL1 A1) loaded EVs	Not open to public	Phase 1
2	Anti-Aging	NCT05813379	Mesenchymal Stem Cells Derived Exosomes in Skin Rejuvenation	01–02–2022	30-08-2023	MSC-Exosome	MSC	Phase 1, phase 2
3	Osteoarthritis	NCT06466850	Mesenchymal Stem Cells Derived Exosomes in Osteoarthritis Patients	01-01-2024	25-01-2025	MSC-Exosome	MSC	Not Applicable
4	Osteoarthritis	NCT05060107	Intra-articular Injection of MSC-derived Exosomes in Knee Osteoarthritis (ExoOA-1) (ExoOA-1)	05–10–2021	05–10–2023	Intra-articular injection of exosomes derived from allogeneic mesenchymal stromal cells	MSC	Phase 1
5	Osteoarthritis	NCT06431152	Intra-articular Injection of UC-MSC Exosome in Knee Osteoarthritis (EXO-OA01)	01–06–2024	31-12-2025	Intra-articular injection of exosomes derived from UC-MSC	UC-MSC	Early phase 1
6	Osteoarthritis	NCT06463132	Phase 1b Clinical Trial to Evaluate PEP and EUFLEXXA for Knee Osteoarthritis (KOA)	01–11–2024	01–11–2025	Intra-articular injection of Purified Exosome Product	Not open to public	Phase 1
7	Osteoarthritis	NCT04223622	Effects of ASC Secretome on Human Osteochondral Explants (ASC-OA)	12-04-2021	12/2024	Secretome of mesenchymal stem/stromal cell (MSC)	Adipose-derived stromal cell	Not Applicable
8	Alzheimer disease	NCT04388982	the Safety and the Efficacy Evaluation of Allogenic Adipose MSC-Exos in Patients With Alzheimer's Disease	01–07–2020	08/2022	Allogenic adipose MSC-derived exosome	ADSC	Phase 1, phase 2
9	Alzheimer disease	NCT05163626	Combined Aerobic Exercise and Cognitive Training in Seniors at Increased Risk for Alzheimer's Disease	12/2024	12/2034	combination of blood neuro-exosomal protein	Blood	Not Applicable
10	Parkinson's disease	NCT05320250	Saliva and Extracellular Vesicles for Parkinson's Disease (RaSPiD)	15/12/2021	31-12-2024	Raman Analysis of saliva and saliva-derived EV	Saliva	Not Applicable
11	Parkinson's disease	NCT05902065	Effect of a Progressive Treadmill Training Protocol for Parkinson's Disease	06/07/2022	31-12-2023	Raman Analysis of blood-derived EV	Blood	Not Applicable
12	Parkinson's disease	NCT03775447	Fox BioNet Project: ECV-003	23/04/2019	18-12-2019	LRRK2, p1292 LRRK2, Rabs, and pRabs expression in CSF-derived EV	Cerebrospinal fuid	Completed

Table 1 (continued)

No.	Conditions	CT ID	Title	Study start	Study completion	Feature	Donor cell	Phase
13	Parkinson's disease	NCT01860118	LRRK2 and Other Novel Exosome Proteins in Parkinson's Disease	01/2013	21-06-2016	LRRK2 expression in exosomes	Brain	Completed
14	Alopecia	NCT05658094	Exosome Effect on Prevention of Hairloss	01–08–2022	29-10-2023	MSC-Exosome	Human Amniotic mesenchymal stem cells	Not Applicable
15	Androgenetic Alopecia	NCT06539273	Exosome Treatment in Androgenetic Alopecia	01-01-2024	05-05-2024	Injection of foreskin-derived Mesenchymal stromal cells-derived exosome	Foreskin-derived MSC	Completed
16	Androgenetic Alopecia	NCT06482541	Efficacy and Safety Of AGE ZERO™ EXOSOMES To Treat Men and Women With Androgenetic Alopecia	07/2024	08/2025	Microneedling of Wharton Jelly's MSC-derived exosome	Wharton Jelly's MSC	Phase 1
17	Androgenetic Alopecia	NCT06239207	Efficacy and Safety of Exosomes Versus Platelet Rich Plasma in Patients of Androgenetic Alopecia	21-09-2023	21-07-2024	Exosomes vs Platelet risch plasma	MSC	Phase 2
18	Age-related Macular Degeneration	NCT06883461	Association Between Age-related Macular Degeneration and Cognitive Impairment Based on Exosome Proteomics	01–04–2019	03–06–2024	Exosome proteomics	Serum	Completed
19	Breast Cancer	NCT05798338	Characterization of Extracellular Vesicles in Breast Cancer Patients	01–12–2020	30-09-2025	Characterization of Plasma EV in breast cancer microenvironment	Tumor	Not Applicable
20	Breast Cancer	NCT05831397	Extracellular Vesicles in Breast Cancer Patientsin Undergone Neoadjuvant Chemotherapy	11-05-2021	21-12-2027	Characterization of Plasma EV in breast cancer microenvironment	Tumor	Not Applicable
21	Breast Cancer	NCT05955521	Exosome as the Prognostic and Predictive Biomarker in EBC Patients	01–05–2021	01–07–2028	exosome evaluation in EBC treated with neoadjuvant chemotherapy	Tumor	Not Applicable
22	Cancer	NCT03262311	Pimo Study: Extracellular Vesicle-based Liquid Biopsy to Detect Hypoxia in Tumours	10–11–2017	12-09-2019	Hypoxia detection through liquid biopsy from EV secreted in blood	Tumor	Not Applicable
23	Meningioma	NCT06104930	Plasma Extracellular Vesicles in Meningioma Patients (MOLI)	01–11–2023	01–02–2026	DNA methylation profiling from EV secreted by cancer cells in blood	Tumor	Not Applicable
24	Retinoblastoma	NCT04164134	New Strategies to Detect Cancers in Carriers of Mutations in RB1 (NIRBTEST)	13-12-2018	31-03-2023	Non-invasive cancer test from EV secreted by tumor cells in blood	Tumor	Not Applicable

Table 1 (continued)

No.	Conditions	CT ID	Title	Study start	Study completion	Feature	Donor cell	Phase
25	Gastrointestinal Cancer	NCT06278064	Exosome-based Liquid Biopsies for Upper Gastrointestinal Cancers Diagnosis	01–02–2024	30-06-2025	Exosomes as biomarker for cancer	Tumor	Not Applicable
26	Gastric Cancer	NCT06342427	Stomach Cancer Exosome-based Detection (DESTINEX)	15-03-2023	15-06-2024	Blood-derived exosomes as biomarker for cancer	Tumor	Not Applicable
27	Pancreas Cancer	NCT03608631	iExosomes in Treating Participants with Metastatic Pancreas Cancer with KrasG12D Mutation	27-01-2021	30-04-2025	iExosomes	MSC	Phase I
28	Lung Cancer	NCT01159288	Trial of a Vaccination With Tumor Antigen-loaded Dendritic Cell-derived Exosomes (CSET 1437)	19-05-2010	19-12-2015	Vaccination With Tumor Antigen-loaded Dendritic Cell-derived Exosomes	Dendritic Cell	Phase II

therapies, it is crucial to develop disease-specific efficacy markers. These indicators, such as total effector molecule expression, the proportion of positive EVs, and the number of EVs carrying specific functional cargo, should be directly linked to the therapeutic relevance of the target disease. The link between these markers and therapeutic efficacy must be validated through rigorous experimental approaches, including dose–response studies, as well as loss-of-function and gain-of-function analyses, to ensure the reliability and consistency of EV formulations.

Another significant drawback of EV-based therapies clinical translation is the divergent regulatory frameworks across nations pertaining to EV-based therapies, which engender substantial challenges in their development and implementation. In the USA and Europe, they classify EVs based on their physiological function, whereas in Asian countries (Japan, South Korea, and Taiwan), the classification system is based on the obtained method [274]. The evident discrepancies in regulatory frameworks underscore the necessity for a universally recognized regulatory standard, particularly to ensure the efficacy and safety of EV-based treatments.

Improving EV detection and quality control

Ensuring the safety, efficacy, and dosage for EV therapy. While many studies have discovered the therapeutic potential of EVs, most have failed to explain the molecular mechanisms by which EVs influence disease development. The development of single-vesicle detection technologies could effectively identify the effector EV subsets within promiscuous systems. Emerging technologies, such as nano-flow cytometry (nanoFCM), offer potentials in improved conclusive detection of EVs. NanoFCM single-particle analysis, providing richer information regarding EV size distribution, concentration, purity, and marker expression compared to other methodologies like Nanoparticle Tracking Analysis (NTA) [275, 276]. Notably, nanoFCM has a lower detection limit than NTA, with the capability to detect particles as small as 40 nm, while NTA is typically limited to particles around 50 nm or larger [277, 278]. This enhanced sensitivity allows for the detection of smaller EVs, such as sEVs. As the development of EV detection technology accelerates and its accuracy improves, it becomes feasible to conduct in-depth study of the mechanisms underlying EV therapy, thereby enhancing our understanding of its therapeutic potentials.

The process of obtaining EVs also lacks standardized quality control measures, including variations in source cells, cell types, culture systems, cell treatment protocol, and isolation techniques. There is a need for consensus on marker identification and efficacy evaluation indicators. In addition, there is limited experimental evidence linking different formulation types (e.g. lyophilized powders, gels, and solutions), or various administration routes (e.g. topical application, intravenous injection, subcutaneous injection, and microneedling) with their therapeutic potency [279, 280]. In terms of safety and efficacy, EVs derived from the same source are often used for multiple indications, and conversely, EVs from various sources often used for the same indication, without robust high-throughput detection of EV content. This leads into huge challenges, including incomplete understanding of EV's active ingredients and mechanism of action. Moreover, the current evidence supporting EV effectiveness is predominantly based on preclinical animal studies, with scarcity of randomized, double-blind clinical trials. Existing clinical trials also face challenges due to small patients' cohorts and limited robust case support. To expedite EV's clinical translation, it is essential to implement comprehensive quality evaluation, ensure process consistency, and establish clear efficacy markers. At the same time, safety must remain a priority, as cell-free therapies do not inherently simplify safety evaluations [10, 14]

Enhancing EV-based therapies through modification

Although EV-based therapy has been proven effective in ameliorating disease progression, it is important to improve the quality, effectiveness, and safety of EV-based therapy. Therefore, several strategies were used to modify EVs, namely through surface modification, cargo loading strategies, and cell programming (Fig. 7).

EV protein surface modification

EVs naturally exhibit selective targeting capabilities, their surface proteins can be engineered to enhance their specificity [175]. Targeted modifications ensure that EVs are internalized only by designated, improving stability and therapeutic precision. Two methods exist for modifying the surface of EVs: exogenous (via click chemistry and physical approaches) and endogenous (via genetic modification) [281, 282]. In click chemistry, alkyne groups react with the EV surface via a condensation reaction of EDC-NHS. A covalent bond forms between the alkyne and azido groups of the EV under copper's presence [281]. A study sought to modify vesicles using this method to target therapy to the brain. The c(RGDyK) peptide was attached to the vesicles'surface, and its effectiveness in a rat MCAO model was evaluated. The results showed that the intravenous injection of cRGD-Exo led to targeted accumulation in brain lesions [283]. Conversely, physical approaches of EV functionalization consist of methods such as sonication, extrusion, and freeze-thawing that can alter the surface properties of EVs through membrane rearrangement [284].

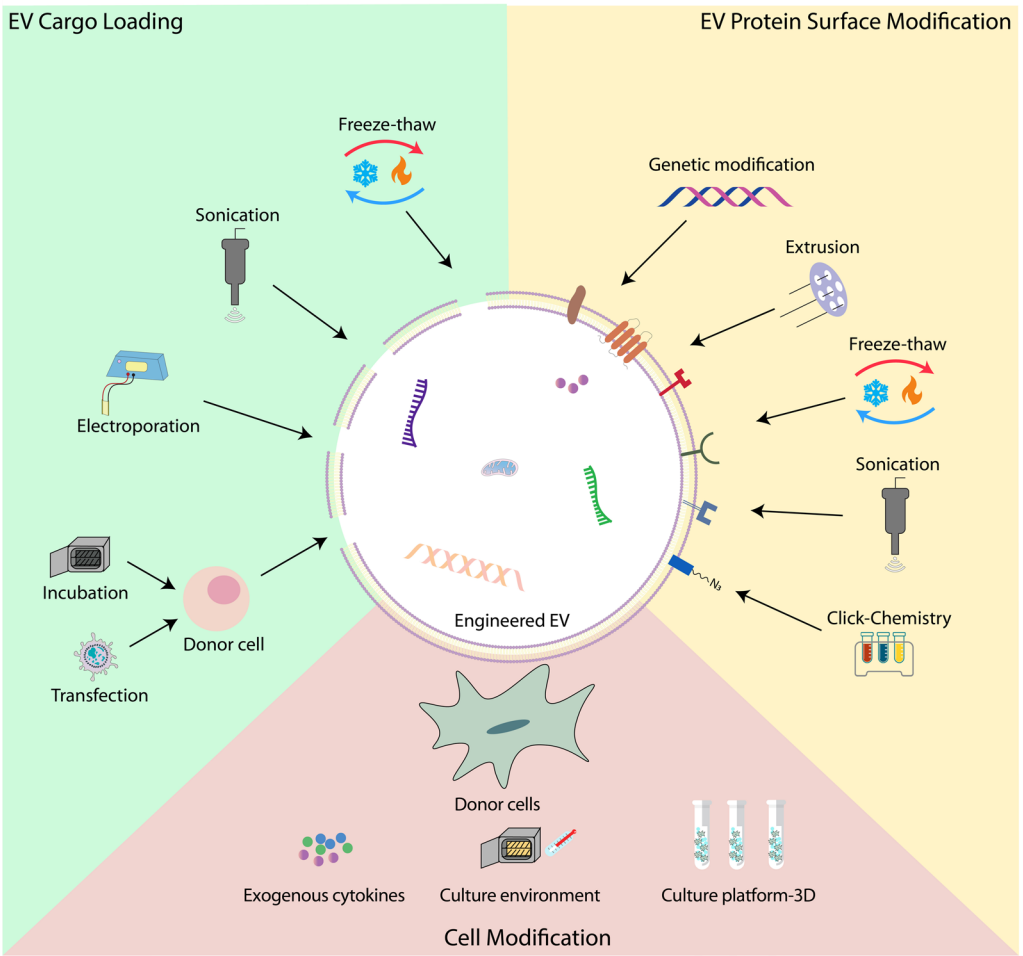


Fig. 7 Strategies for engineering EVs. Engineered EVs represent a promising approach to enhance their therapeutic potential. Various strategies, such as cell donor modification, EV protein surface modification, and specific cargo loading, have been extensively studied, and shown to improve EV's targeting ability, stability, and scalability

Endogenous methods are widely used to modify the surface of EVs. For instance, CD47 modification on EVs'surface proteins enhances stability and facilitates internalization by target cells. A recent study showed that CD47-modified EVs'half-life in plasma increased from less than 30–120 min [285]. CD47 modification has also been observed to reduce the phagocytosis of EVs by macrophages, prolonging the circulation time of EVs in plasma [286]. The fusion of a molecule with EVs'surface proteins is a common EV modification method [287, 288]. This has been observed in mice, with modified EVs demonstrating longer circulation times after injection and attributed to the enhanced binding and local release capability of EVs. Additionally, they demonstrated enhanced accumulation in lymph node and solid lesions accumulation, improving the accessibility of EVs in immunotherapy [289]. The fusion of Apolipoprotein A-1 and CD63 on the surface of secreted exosome facilitates selective binding to HepG2 cells [290]. In addition to entire proteins, the fusion of T7-peptide with Lamp2b has shown improved efficacy in treating glioblastoma using exosome-based methods [291]. This modification has been shown to enhance the ability of exosome to traverse the BBB, a crucial step in advancing drug-delivery therapy for brain-related conditions.

Biomaterials bind with lipid anchors of EV membrane also enhance the selectivity and specificity of EVs. Polymer (polyoxazoline) addition to EVs'surface modification increases the circulation time of EVs in plasma, increasing the half-life up to 6 h after injection and increasing the accumulation of EVs in pancreatic tumor [292]. Polymer-modified EVs do not accumulate significantly in the spleen and kidney, suggesting that internalization of EVs in pancreatic tumors is highly effective. Developing EV-targeted therapies for tumor cells requires considering the adverse effects of modified EVs and the stabilization,

targeted-therapy ability, and distribution of EVs [292]. Conducting in-depth research to develop an effective EV modification method that enhances the therapeutic potential of EVs without compromising the safety profile of EV-based treatments is imperative.

EV cargo loading

In addition to surface protein engineering, the encapsulation specificity of EVs can be optimized. Two approaches have been developed for incorporating specific payloads on EVs. The first approach involves the incorporation of payloads on EV donor cells so that secreted EVs will carry the targeted molecules (endogenous loading). The second approach involves the incorporation of payloads directly on EVs that have been isolated first (exogenous loading) [282]. Endogenous methods have been shown to be superior for the loading of macromolecules, including proteins, polymers, and drugs. In this method, the most commonly used techniques are incubation and transfection.

Conversely, exogenous methods have been shown to exhibit high efficiency; however, their applicability is limited to the loading of smaller molecules, including siRNAs, miRNAs, proteins, CRISPR/Cas9 systems, natural products derived from hydrophobic compounds, and anticancer drugs [293, 294]. However, the proper choice of cargo loading technique is critical, as some methods can compromise the stability and integrity of EVs, potentially reducing therapeutic efficacy. Modifications to the preparation steps or the addition of stabilizing reagents can help maintain EV function while improving their therapeutic performance [179].

Cell programming escalates EVs therapeutic potential

Modifications to the parental cell line can lead to phenotype and secretome changes in EVs. Strategies such as stem cell priming have been studied and shown to improve the therapeutic effect of EVs. Priming of MSCs can be done through three approaches; priming through exogenous cytokines, culture environment and culture platform [295]. Priming MSCs through inflammatory molecules produces EVs that carry proteins related to stimulate cell motility and modulate inflammatory and degenerative processes. This is consistent with what was obtained by Ragni et al., after studying the effect of inflammatory priming on ADSCs under IFNγ stimulation [296]. In addition, ADSCs cultured under hypoxia conditions are also known to secrete exosomes that are more easily internalized by endothelial cells, and induce cell angiogenesis ability through activation of the protein kinase A pathway [297]. Similar effects were also shown by EVs secreted by MSCs using 3D culture (Spheroid). This culture platform enhances the therapeutic ability of EVs, especially in diseases that require angiogenesis processes, such as alleviating skin aging and wound healing. In addition to being pro-angiogenic, these EVs were also observed to have antioxidant abilities in mouse liver cells and human liver epithelial cells, through overexpression of antioxidant genes, such as SOD1, SOD2 and NRF2 [298].

The role of EVs in personalized medicine

Personalized medicine is an emerging approach that tailors disease prevention, diagnosis, and treatment based on an individual's genetic, molecular, physiological, behavioral, and environmental characteristics [299]. Because disease progression varies between individuals, EVs play a key role as information carriers, reflecting the state of their originating cells.

Based on emerging research, we propose following applications of EVs in precision therapy: First, as mentioned in the "Caner" section, EV-specific miRNAs or protein profiles can be used for cancer early screening, tumor classification, and prognosis evaluation [300]. Additionally, EVs offer potential in cancer vaccine development. For example, a novel boron neutron capture therapy enhances the DNA content in tumor cell-derived EVs by promoting DNA breaks and the degradation of DNA antioxidant enzymes. When internalized by dendritic cells, these EVs leads to stronger antitumor effects and boosts the potential of dendritic cell-based vaccines [301]. Moreover, EVs can dynamically monitor treatment responses by tracking therapeutic outcomes through minimally invasive sampling of body fluids such as blood or urine. The liquid biopsy of urinary and plasma EVs has good diagnostic ability for injury and drug responses [302–304]. At the same time, EV-based bioanalysis is driven by emerging digital bioassays, microfluidic Topographically Intensified, Partition-less dELISA (μTIP-dELISA) is a single-molecule signal amplification strategy for EV markers detection, confering more than 300-fold improvement over the conventional ELISA for biomarkers and exhibiting highly sensitive and specific detection capability [305]. Furthermore, integrating EV proteomics and miRNA data with machine learning algorithms enables the prediction of treatment sensitivity, optimizing therapeutic strategies. Zhang et al. performed a fecal extracellular vesicle microRNA signature (FEVOR), which revealed by miRNA sequencing with machine learning-driven models. This FEVOR diagnostic model achieved an outstanding accuracy of 97.4% [306]. Overall, these applications highlight EVs as versatile tools in advancing precision medicine.

Summary and discussion

Between 2015 and 2050, the global population aged over 60 years is projected to double from 11 to 22% [4]. This demographic number is expected to be followed by an increase in age-related diseases, causing significant socioeconomic burden on healthcare system. Therefore, improving healthcare system quality, and supporting healthy aging is greatly important.

Extracellular vesicles (EVs) have emerged as promising therapeutic tools especially, in cargo or drug-delivery. These double membrane structures are secreted by the cells and function as cell-to-cell messengers, carrying a variety of molecular cargo such as proteins, nucleic acids, lipids, and organelles [15, 16]. The cargo within EVs is protected by lipid bilayer surface, protecting it from degradation caused by harsh extracellular environment [307]. These biocargoes often to be involved in certain pathways associated with the pathogenesis of age-related diseases. Following this discovery, researchers have begun to delve deeper into EVs' potential in both understanding disease pathogenesis as well as developing therapeutic applications.

In this review, we discuss the role of EVs in the pathogenesis, therapeutic ability and diagnosis of age-related diseases. We found that, in many age-related diseases, EVs function as a double-edged sword; they contribute to worsen the disease, however the miRNA, proteins and mitochondrial components they carry also exhibit therapeutic potential. In addition, EVs secreted from senescent cells tend to contain elevated levels of SASP and pro-inflammatory cytokines, or abnormal levels of certain protein. Detecting these molecules may allow for early diagnosis of age-related diseases. Eventually, this facilitates patient for timely treatment, reducing mortality rates, and alleviating the burden caused by the diseases.

Although EVs have demonstrated considerable promise as delivery vehicles for the treatment of age-related diseases, the FDA has not yet approved any EV-based therapies. The complexity of EVs' mechanisms in disease pathogenesis and lack of standardized isolation and purification methods, has hindered regulatory approval. Implementing proper quality control measures and developing EV modification techniques may serve ways to tackle these problems. In addition, future studies are needed to create a comprehensive atlas of the mechanisms underlying EV-based therapies.

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Author contributions
PHLP and YF wrote the paper; PHLP drew the figures and tables; YF, SHA, and XD revised the paper.

Data availability
No data was used in the article.

Declarations

Competing interests
The authors declare that they have no competing interests.

Declaration of generative AI in scientific writing
During the preparation of the manuscript, the authors used Perplexity AI to enhance the readability and clarity of the text, as well as to reduce grammatical errors. This tool assisted in ensuring that the manuscript's statements were presented clearly and understandably, facilitating better communication of the research findings. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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