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REVIEW ARTICLE

Roles of extracellular vesicles in the aging microenvironment and age-related diseases

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Abstract

Cellular senescence is a persistently hypoproliferative state with diverse stressors in a specific aging microenvironment. Senescent cells have a double-edged sword effect: they can be physiologically beneficial for tissue repair, organ growth, and body homeostasis, and they can be pathologically harmful in age-related diseases. Among the hallmarks of senescence, the SASP, especially SASP-related extracellular vesicle (EV) signalling, plays the leading role in aging transmission via paracrine and endocrine mechanisms. EVs are successful in intercellular and interorgan communication in the aging microenvironment and age-related diseases. They have detrimental effects on downstream targets at the levels of immunity, inflammation, gene expression, and metabolism. Furthermore, EVs obtained from different donors are also promising materials and tools for antiaging treatments and are used for regeneration and rejuvenation in cell-free systems. Here, we describe the characteristics of cellular senescence and the aging microenvironment, concentrating on the production and function of EVs in age-related diseases, and provide new ideas for antiaging therapy with EVs.

KEYWORDS

age-related diseases, aging microenvironment, antiaging therapy, cellular senescence, extracellular vesicles

1 | INTRODUCTION

Cellular senescence is a physiologically stable state of cell cycle arrest that strictly regulates cell proliferation and lifespan. The discovery of this phenomenon can be traced to the 1960s. Hayflick and Moorhead cultivated human fibroblasts and observed that they underwent a limited number of cell divisions, which is now called the "Hayflick limitation" (Hayflick & Moorhead, 1961). Cellular senescence is a transitional state of cell death that precisely regulates survival and death at the cellular level. The expected cellular senescence trajectory is cell elimination, which plays an essential role in promoting tissue repair and regulating organ growth and body homeostasis (Mosteiro et al., 2016; Ritschka et al., 2017). However, in senescence-associated diseases, excessive accumulation of senescent cells during aging becomes burdensome, disrupting the trajectory of programmed cell death and leading to a decline in regeneration (Masaldan et al., 2018). These findings indicate that short-term accumulation of senescent cells in tissues is beneficial to the renewal of the internal environment and the maintenance of organs but that long-term accumulation of senescent cells in tissues triggers an imbalance in the internal environment and organ disability.

This double-edged sword effect of cellular senescence is specifically attributed to the senescence-associated secretory phenotype (SASP), which can be beneficial by inducing the recruitment of immune cells against stressors and can be detrimental by inducing the transmission of aging signals to cause inflammation (Ovadya et al., 2018). Among these secretory factors, extracellular vesicles (EVs), diverse small vesicles with lipid bilayer membranes, are released by most cell types and have been detected in virtually all body fluids (Saugstad et al., 2017). Their omnidirectional communication among cells and organs indicates their

HIGHLIGHTS

- The aging microenvironment can be unstable with aging and transform into a pathological state.
- The SASP, with the involvement of EVs, amplifies senescent signals in autocrine, paracrine, and endocrine ways.
- Senescent EVs alter the levels of immunity, inflammation, gene expression and metabolism of target cells.
- Therapeutic strategies incorporating EVs from various materials and donors are feasible for age-related diseases.

tight association with the transmission of aging signals in the context of age-related diseases. Transmission through EVs can induce immune and inflammatory activation, genomic instability, telomere attrition, epigenetic alterations, mitochondrial and lysosomal system dysfunction, ROS accumulation, loss of proteostasis and nutrition, and stem cell exhaustion in target cells. Their potential for clinical application suggests that they can be used therapeutically to extend lifespan. In this review, we focus on the role of EVs in the aging microenvironment and age-related diseases and the value of EVs as biomarkers and treatments of pathological aging states.

2 | EXTRACELLULAR VESICLES IN THE CELLULAR SENESCENCE AND AGING MICROENVIRONMENT

2.1 | Cellular senescence and the aging microenvironment

From a longitudinal perspective, single-cell aging can be considered a fate-decision process according to Li's single-cell analysis of yeast performed with microfluidics and time-lapse microscopy. Although cells carrying identical genetic materials were cultured in uniform medium, they embarked on two distinct paths of aging, bifurcating early: one pathway led to rDNA silencing and nucleolar decline, and the other led to heme abundance and mitochondrial aggregation (Li et al., 2020b). The findings pinpointed two essential events of cellular senescence: changes in nuclear genes and the transformation of mitochondria (Figure 1).

As mitochondria are crucial double-membrane organelles in the progression of the tricarboxylic acid (TCA) cycle and electron transport chain, the production of ATP, the synthesis and breakdown of lipids and the regulation of apoptosis, they are regarded as the key drivers and regulators of senescence (Habiballa et al., 2019). They undergo biogenesis, dynamic fusion and fission, and mitophagy to ensure structural and functional homeostasis in response to stimuli and stresses, and disruption of any of these steps can cause mitochondrial dysfunction and detrimental oxidative metabolism leading to cellular senescence (Picca et al., 2018). The role of mitochondrial biogenesis in aging has been controversial. Mostly, pathologically senescent cells have been shown to exhibit increased mitochondrial mass with increasing oxygen consumption and ROS production by transcriptional coactivator PGC-1, which is a regulator of mitochondrial biogenesis (Correia-Melo et al., 2016). However, senescent $CD4^+$ T cells display a higher mitochondrial content and biogenesis than $CD8^+$ T cells, while retaining better glucose and lipid uptake, nutrient usage, and proliferative and migratory capacity (Callender et al., 2020). The significance of mitochondrial dynamics has been shown in a *Pgam5* deficiency mouse model in vivo and *PGAM5*$^{-/-}$ cells in vitro (PGAM5 is a mitochondrial Ser/Thr phosphatase that dephosphorylates Drp1 and promotes mitochondrial fission). In these models, reduced mitochondrial fission increased ATP and ROS levels, elevated signaling via mTOR and IFN pathways and resulted in an age-related reduction in antioxidation capability (Yu et al., 2020). Like mitochondrial fission and fusion, mitophagy is a selective process of mitochondrial quality control; however, mitophagy determines dysfunctional mitochondrial degradation by lysosomes. In yeast, defects in lysosome-like vacuoles induce intracellular cysteine toxicity, collectively hampering iron bioavailability and mitochondrial respiration via a ROS-based mechanism (Hughes et al., 2020). Altogether, these data indicate that impaired mitochondrial homeostasis may result in elevated ROS levels and senescence. In addition to elevated ROS levels, senescence-associated mitochondrial metabolism can be characterized by reduced NAD+/NADH ratios, abundance of metabolites, release of mitochondrial damage-associated molecular patterns (DAMPs) and mutation of mtDNA (Habiballa et al., 2019).

Accordingly, several studies have indicated that the accumulation of ROS is not a simple consequence of cellular senescence but also a trigger and accelerator of DNA damage and aging (Bai et al., 2020; Jia et al., 2014; Qian et al., 2019; Yin et al., 2013). The presence of DNA damage activates a robust response named the DNA damage response (DDR) (Calcinotto et al., 2019). Common DDR outcomes realized from intrinsic insults include telomere shortening (Azarm et al., 2020), oncogene mutation (Hafez et al., 2017), and oxidative damage (Oh et al., 2017). Various DNA-damaging agents are also effectively applied to induce different types of senescence, for example, chemotherapy-induced senescence (e.g., that induced by doxorubicin (Yao et al., 2020) or etoposide (Zurgil et al., 2014)). The DDR activates the ATM/ATR pathway to block cell cycle progression via stabilization of the p53 protein (Stewart-Ornstein & Lahav, 2017) or alternative splicing of TP53 mRNA (Chen et al., 2017), which then stimulates cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ transcription (Thurn et al., 2013). The p21 signal is essential at the onset

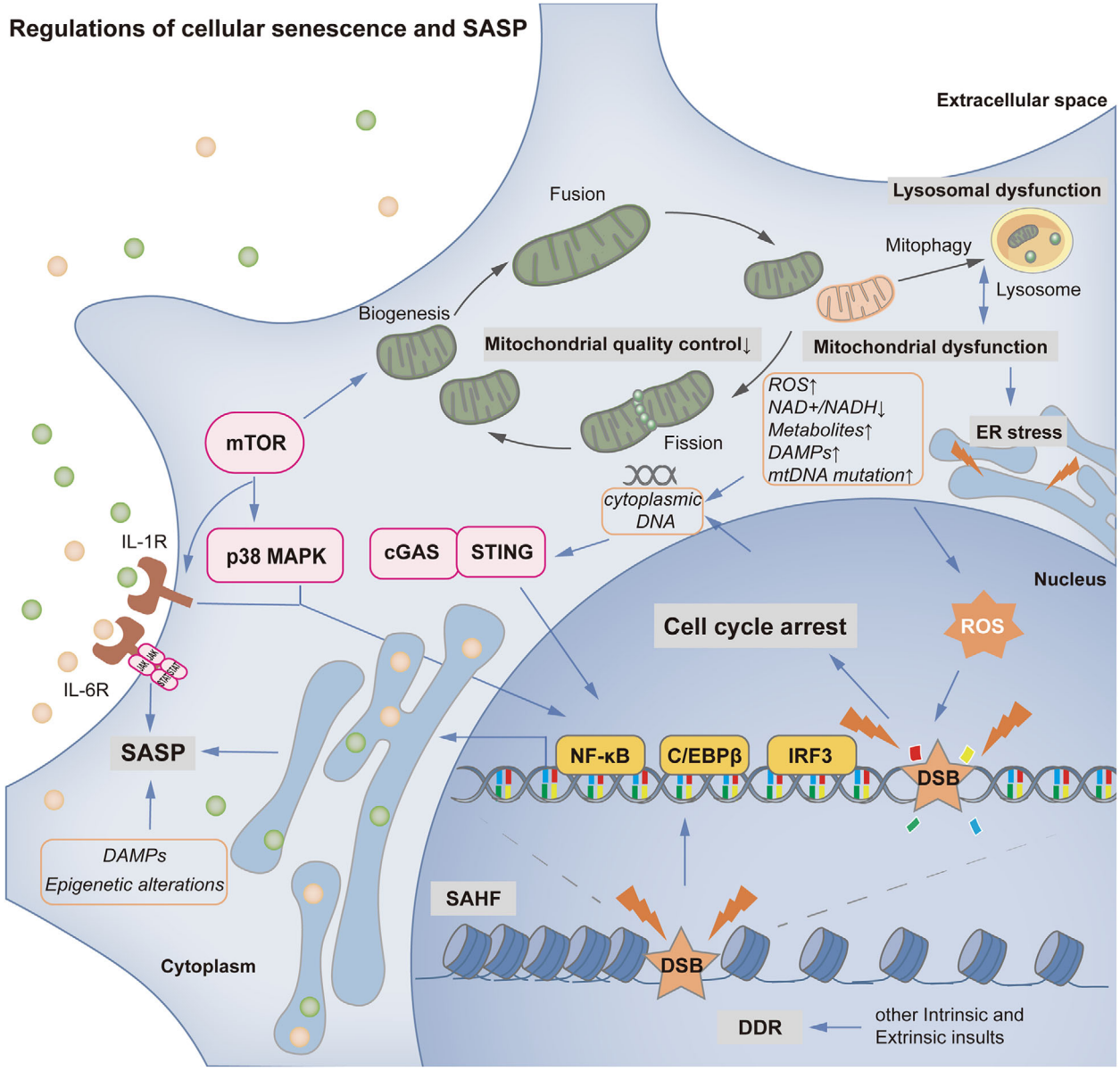

FIGURE 1 Regulation of cellular senescence and SASP. In cellular senescence, there are two essential events: changes in nuclear genes (associated with DDR, SAHF and other epigenetic alterations) and the transformation of mitochondria (unbalanced mitochondrial quality control and mitochondrial dysfunction). Mitochondrial changes, which may be induced by lysosomal dysfunction and mTOR signaling, can result in elevated ROS levels, reduced NAD+/NADH ratios, increased abundance of metabolites, release of DAMPs, mutation of mtDNA, lysosomal dysfunction and ER stress. The accumulation of ROS can trigger and accelerate DNA damage and cell cycle arrest. In addition to ROS, many intrinsic and extrinsic insults can also trigger DDR and cell cycle arrest. In the SASP process, several signaling pathways (including mTOR, p38 MAPK, cGAS/STING, DDR/PIKK superfamily, epigenetic alteration, and DAMP pathways) are involved. Downstream transcription factors, such as NF-κB, C/EBP*β* and IRF3, are consistently recruited to regulate SASP-related gene expression. Functionally, the SASP can transmit aging signals in autocrine, paracrine, and endocrine ways. Hallmarks of senescence are shown in grey. Controlling productions or events in aging are shown in orange. Important signaling pathways are shown in pink. Essential transcription factors are shown in yellow. Receptors are shown in brown

of cell cycle arrest, while it is replaced by the subsequently increasing p16 level, both of which can prevent Rb phosphorylation to slow cell division in aging (Figure 2) (Prasnikar et al., 2021). Moreover, the retrograde signal between mitochondria and the nucleus impacts nuclear gene expression in the direction of senescence. In an aged mouse model, the RNA component of telomerase *TERC* was found to be imported into mitochondria and then exported to the cytosol as a short form, *TERC-53*, which reveals mitochondrial function and induces cognitive decline by changing nuclear gene expression (Zheng et al., 2019). Most genes can also display changes in the epigenome and chromatin organization, typically at senescence-associated heterochromatin foci (SAHF), to drive aging. These changes can be triggered by disruption of mitochondrial function, glucose metabolism and autophagy (Di Micco et al., 2021; Zhang et al., 2021).

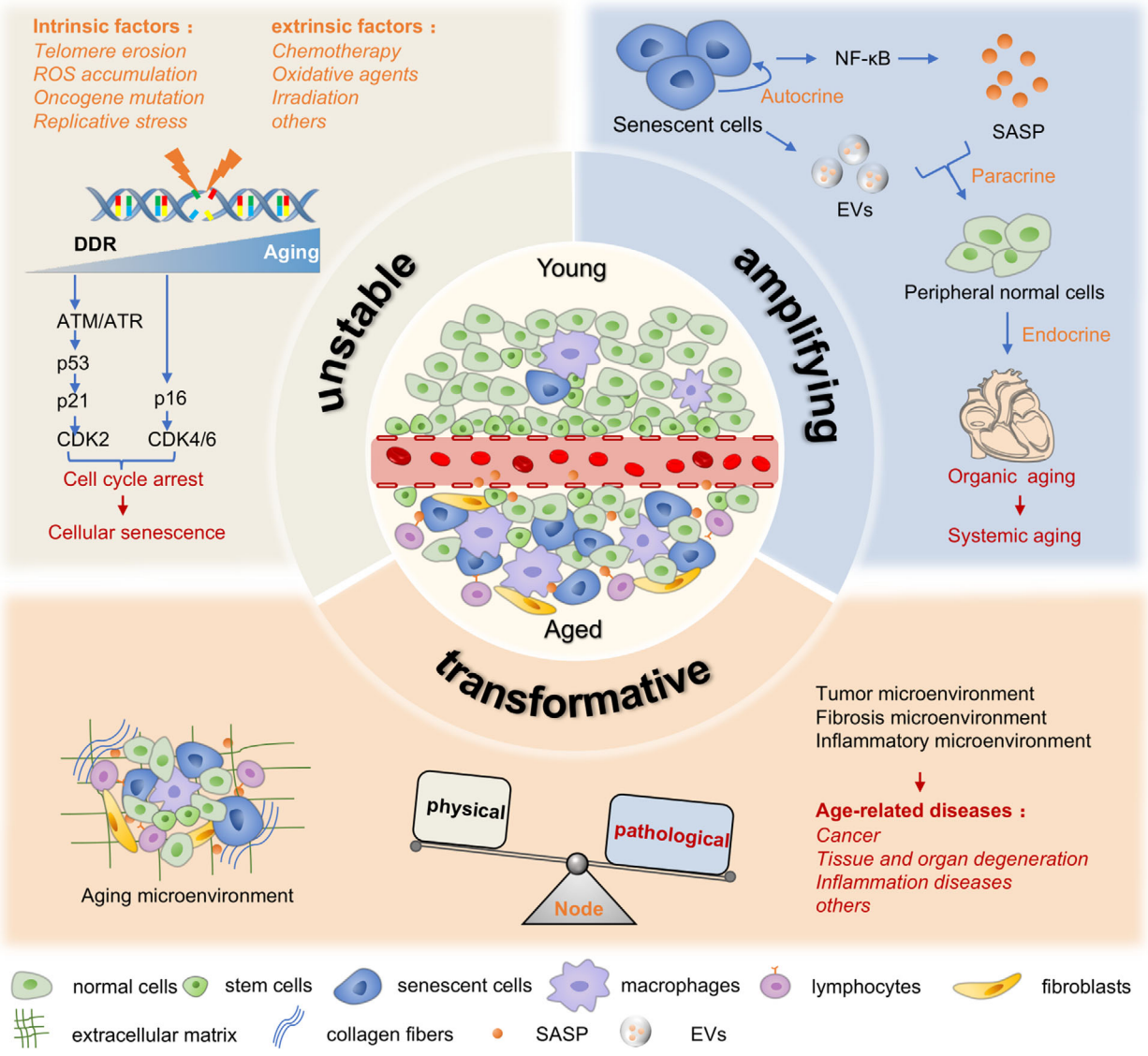

FIGURE 2 Three characteristics of the aging microenvironment. The aging microenvironment contains fewer proliferative stem cells and more senescent functional cells and immunosuppressive cells with age. It is increasingly unstable because of intrinsic and extrinsic stressors, leading to the DNA damage response and cell cycle arrest. The SASP, which includes EVs, can amplify senescent signals from accumulating senescent cells to peripheral normal cells or distant organs via autocrine, paracrine and endocrine pathways. The aging microenvironment can be transformative and reprogrammed through specific nodes. The physical microenvironment can be transformed into pathological tumours, fibrosis and inflammatory microenvironments, inducing age-related diseases, such as cancer, degenerative diseases and inflammation

However, in a special type of senescence, PTEN loss-induced senescence (PICS), mammalian target of rapamycin complexes 1 and 2 (mTORC1 and mTORC2) can directly stabilize p53 and initiate the p21-associated senescent pathway through PI3K/AKT activation and MDM2 inhibition without triggering the DDR (Jung et al., 2019). The mTOR signaling pathway also controls mitochondrial activities to regulate energy metabolism and oxidative phosphorylation progress. It has been documented that mTORC1 can promote nuclear-encoded mitochondria-related gene expression through inhibition of eukaryotic translation initiation factor 4E (elF4E)-binding proteins (4E-BPs) or interaction with PCG-1α, consequently increasing mitochondrial biogenesis (de la Cruz Lopez et al., 2019; Morita et al., 2013). Simultaneously, mTORC1 can selectively stimulate the translation of mitochondrial fission process 1 (MTFP1) via 4E-BPs, recruit the fission GTPase dynamin-related protein (DRP1) and govern mitochondrial dynamics (Morita et al., 2017). In multiform senescence, mTORC1-related mitochondrial biogenesis can activate the ROS-induced DDR through the ATM/Akt/mTORC1 phosphorylation cascade and then upregulate PCG-1β, which has impacts on senescent deterioration and SASP persistence in vitro and in vivo (Correia-Melo et al., 2016). In addition, mTOR signaling in aging also interferes with protein metabolism and blocks autophagy and regeneration to accelerate cellular and tissue senescence (Liu & Sabatini, 2020).

With mitochondrial dysfunction, elevated ROS levels can also induce endoplasmic reticulum (ER) stress via unfolded protein response (UPR) signaling, in which FGF21 as the culprit systemically triggers premature senescence (Tezze et al., 2017). Whereas mitochondria suffer mitophagy and dysfunction, lysosomes undergo deterioration with the accumulation of lipofuscins (Park et al., 2018). In this vicious feedback cycle, autophagic flux also consequently shows impaired proteolytic ability, and this impairment is responsible for chronic senescence (Tai et al., 2017). Thus, due to the mechanistic complexity and intersection of the causes and types of single-cell senescence, the currently known senescent hallmarks do not have specificity.

At eye level, senescence does not merely affect a single group of cells. Senescence is always the consequence of the coaction of different cells, cytokines, extracellular matrix components, and other elements. This combination is regarded as the aging microenvironment and is mainly composed of proliferating cells, functional and metabolic cells, and surveillance and elimination factors (Fane & Weeraratna, 2020). Here, we conclude three characteristics of the aging microenvironment (Figure 2). First, it grows increasingly unstable with age. The body confronts various risk factors over time, which can be external or internal, genetic or acquired, and with sudden and powerful effects or cumulative and weak effects. When systems are disturbed and illness strikes, stem cell exhaustion, metabolic dysfunction, byproduct accumulation, and an imbalance of the microenvironment can emerge, ultimately leading to cancer or other age-related diseases. Fane and Weeraratna indicated that compared to the young, healthy microenvironment, the aging microenvironment demonstrates accumulation of SASP factors and immunosuppressive cells, such as regulatory T cells and myeloid-derived suppressor cells, which accelerate the instability of the aging microenvironment and induce tumour-permissive conditions (Fane & Weeraratna, 2020). A metabolic study on elderly individuals has also confirmed this process. In the serum, the levels of methylmalonic acid, a byproduct of propionate metabolism, increase with age. This metabolic alteration changes the aging microenvironment and favours the epithelial-mesenchymal transition in lung and breast cancer through the TGF-β and SOX4 pathways (Gomes et al., 2020).

Second, the aging microenvironment has an amplifying effect. Senescent cells can transmit aging signals via the SASP involving extracellular vesicles to magnify aging signals among cells and organs (Al Suraih et al., 2020). In ischemic retinal models, senescent retinal ganglion cells, microglia and endothelial cells can release many SASP factors, such as TGF-β, IL-1, IL-6 and VEGF, to reinforce their senescence in autocrine ways and propagate senescent alterations via the paracrine pathway in the whole retina, contributing to pathological retinal angiogenesis (Oubaha et al., 2016).

Finally, the aging microenvironment can be transformative and reprogrammed. With the above characteristics, the aging microenvironment can be easily transformed into a pathological microenvironment. For example, markers in the aging microenvironment can also be detected in the tumour microenvironment (Han et al., 2020). There are even specific controllable nodes in this transformation. Chen et al. confirmed that neuropilin-1 (NRP1) and HIF2α were highly expressed in aging lungs and significantly downregulated endothelial cell protein C receptor (EPCR), a regulator of the circulating microenvironment. Inhibition of the EPCR pathway had important effects on a variety of functions from regeneration to fibrosis in the context of the circulating aging microenvironment (Chen et al., 2020). Selectively inhibiting neuropilin-1 (NRP1) and HIF2α in vascular endothelial cells (ECs) significantly attenuated the fibrosis process, indicating that this research area is fertile ground for targeted therapy development.

These findings show that organ aging in the aging microenvironment is multidirectional and multimembered. Systemic aging is characterized by multiformity associated with alterations and combinations of various molecules, cells, and organs. Based on this theory, individual ageotypes (depicted and formulated as different types of aging patterns in diverse populations) will provide molecular assessment data (including genes, plasma proteins, metabolites, cytokines, and microbes) that precisely reflect personal aging levels (Ahadi et al., 2020).

2.2 | SASP and cellular senescence

Among ageotypes, various soluble molecules marking the presence of senescent cells, which together are termed the SASP, have attracted our attention because of their heterogeneity and availability for clinical application. High levels of immune modulators, proinflammatory cytokines, growth factors, chemokines, and proteases are involved in the SASP in a cell non-autonomous manner (Calcinotto et al., 2019). Proteomic analysis results obtained from human fibroblasts, renal epithelial cells, bone marrow, and adipose mesenchymal stromal cells have suggested that the SASP can be categorized into different groups depending on the senescence inducers and cell derivations (Basisty et al., 2020; Ozcan et al., 2016). In contrast to genotoxic and oncogenic senescence, the work by Wiley et al. presented a distinct senescent phenotype of mitochondrial dysfunction in progeroid mice with $POLG^{D257A}$ mitochondrial DNA (mtDNA) mutation, termed mitochondrial dysfunction-associated senescence (MiDAS), which had a specific secretion profile without IL-1-dependent factors through an NADH/AMPK/p53-dependent pathway (Wiley et al., 2016). Additionally, the SASP Atlas website (www.SASPAtlas.com), constructed by Basisty's team, can aid in a more comprehensive understanding of the complex and dynamic SASP network (Basisty et al., 2020).

Paradoxically, the SASP can be beneficial to organismal homeostasis or detrimental in different biological contexts (Oubaha et al., 2016; Watanabe et al., 2017). When primary mouse keratinocytes are transiently subjected to SASP conditions, the levels of stem cell markers and regenerative capacity increase in vivo. In contrast, under prolonged exposure, cell plasticity and stemness

TABLE 1 Signaling pathways that are associated with SASP-related secretion

Trigger	Signaling pathway	Transcription factor(s)	Secretory factors	Ref.
DNA damage response				
H2A.J accumulation	Incorporation into SAHFs; H1 decrease	STAT, IRF	IL-6, IL-8, CXCL8, CSF2, CM-CSF, MCP1, CCL2, IFN	(Isermann et al., 2020); (Mangelinck et al., 2020)
MLL1 increase	ATM	NF-κB	IL-8, IL-1B	(Capell et al., 2016)
Autophagy decrease	GATA4	NF-κB	IL-6, IL-8, CXCL1/2/3, CM-CSF, ICAM1, CCL2, TNF	(Kang et al., 2015)
Mitochondrial dysfunction				
NAD+/NADH ratio decrease	NADH/AMPK/p53	/	IL-10, TNFα, CCL27	(Wiley et al., 2016)
ROS and CCF increases	cGAS/STING	NF-κB, IRF3	IL-6, CXCL10	(Vizioli et al., 2020)
Cell-free mtDNA	cGAS/STING; TLR9-DC activation	IRF3; T-bet, RORγt	IFN; IL-17, IFN-γ	(Aarreberg et al., 2019); (Iske et al., 2020)
Chromatin remodeling				
LBR decrease	CCFs/cGAS/STING	/	IL-6, IL-8, MMP1	(En et al., 2020)
LINE1 transcription increase	cGAS/STING	NF-κB, IRF3	IFN, IL-6, IL-8, IL-1, MMP3, ISG, MCP1	(Bi et al., 2020)
HDAC inhibition	ATM/MRN	NF-κB	IL-6, IL-8, CM-CSF, IL-1	(Malaquin et al., 2020)
BRD4 increase	Acetylation of superenhancers of the SASP	/	IL-6, IL-8, CM-CSF, IL-1, CXCL1, G-CSF	(Tasdemir et al., 2016)
Other triggers				
Oncogenic Ras increase	MAPK/PEA3/SCCA; P38 MAPK	NF-κB	IL-6, IL-8, CXCL1, G-CSF, CM-CSF	(Catanzaro et al., 2014); (Freund et al., 2011)
HMGA protein increase	NAMPT/NAD+/p38 MAPK	NF-κB	IL-6, IL-8, IL-1B	(Nacarelli et al., 2019)
HMGB1 protein increase	TLR4 signaling	NF-κB	TNFα, IL-6, IL-8,	(Davalos et al., 2013)
Inflammasome increase	IL-1 signaling	/	IL-1, IL-6, IL-8, CCL2	(Acosta et al., 2013)
PTBP1 increase	Alternative splicing of EXOC7	/	IL-6, IL-8	(Georgilis et al., 2018)
S100A14 and Cu^{2+} increases	IL-1 signaling	NF-κB	IL-6, IL-8, IL-1, MMP3, CXCL1/2, CCL2	(Su et al., 2019b)
mTOR increase	IL-1 signaling	NF-κB	IL-6, IL-8, IL-1, CXCL1/2, CCL2	(Laberge et al., 2015)
NOTCH1 decrease	NOTCH	C/EBPβ	IL-6, IL-8, IL-1	(Ito et al., 2017)
DNase2 and TREX1 decrease	CCFs/cGAS/STING	NF-κB, IRF3	IL-6, IL-8, IL-1, CXCL10, IFN	(Takahashi et al., 2018)

are attenuated to some extent (Ritschka et al., 2017). Therefore, it can be assumed that the roles of the SASP can autonomously switch depending on the concentration and duration of treatment. Moreover, high levels of SASP proteins are positively correlated with the age and physical conditions of patients. Among these SASP factors, the levels of 7 SASP factors (GDF15, FAS, OPN, TNFR1, ACTIVIN A, CCL3 and IL-15) may predict the postsurgery outcomes of patients with severe aortic stenosis and ovarian cancer (Schafer et al., 2020).

When the SASP is stimulated, several signaling pathways are activated, and two downstream transcription factors, NF-κB and C/EBPβ, are consistently recruited to regulate SASP-related gene expression (Figure 1 and Table 1). With regard to DNA damage-induced senescence, mounting evidence has indicated that the phosphatidylinositol 3-kinase-related kinase (PIKK) superfamily (ATM, ATR and DNA-PKcs protein kinases included) is crucial for the onset of senescence and transcription of SASP genes upstream of NF-κB signaling, responding to the DDR (Strzeszewska et al., 2018). Pharmacologic inhibition and genetic depletion of ATM can suppress NF-κB activation, diminish inflammatory cytokine levels, and reverse senescence (Zhao et al., 2020). ATM and ATR also inhibit p62-dependent autophagic degradation to stabilize the GATA4 protein, which evokes the NF-κB signaling pathway, thereby contributing to the SASP (Kang et al., 2015). In addition, the transcription-associated methyltransferase and oncoprotein MLL1 also mobilizes the ATM-NF-κB axis to control SASP genes in the context of the DDR (Capell et al., 2016). With a prolonged DDR, the accumulated histone variant H2A.J colocalizes with 53BP1 to be incorporated into SAHF formation and competes with H1 to increase the expression of repeated DNA sequences while activating STAT/IRF transcription factors and inducing the interferon-related SASP (Isermann et al., 2020; Mangelinck et al., 2020).

Epigenetically, bromodomain-containing protein 4 (BRD4), an acetylated histone-binding protein, is directly recruited to superenhancers adjacent to SASP genes, and myriad cytokines are released (Tasdemir et al., 2016). Nevertheless, histone deacetylase inhibitors (HDACis) are required for the recruitment of ATM and MRN complexes, and NF-κB binds to chromatin to modulate the expression of SASP genes, which is independent of DDR (Malaquin et al., 2020). Regarding the sirtuin family, a group of histone deacetylases, their declining expression with age determines the detachment of nuclear lamina proteins and the loss of heterochromatin proteins, such as SIRT7. SIRT7 downregulation promotes the transcription and accumulation of the LINE1 retrotransposon (LINE1), which triggers the innate immune response through the cGAS-STING signaling pathway (Bi et al., 2020). Besides the decrease in SIRT7, the decrease in the laminar protein lamin B receptor (LBR) also causes dysregulation of heterochromatin and generation of cytoplasmic chromatin fragments (CCFs) to activate the cGAS-STING pathway in senescent cells (En et al., 2020).

Thus, the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway is critical for SASP modulation in response to the accumulation of cytoplasmic DNA (CCFs, cDNA and mtDNA). Diverse triggers of senescence (such as oxidative stress, oncogene mutation, irradiation, and pro-senescent drug treatment) can cause extrusion of cytoplasmic DNA and activation of the innate immune sensing cGAS-STING pathway, which next activates TBK1 and IκBα to promote IRF3 and NF-κB expression (Gluck et al., 2017; Loo et al., 2020). Moreover, ROS generated by mitochondrial dysfunction can promote linking of the kinase JNK with 52BP1 (a nuclear protein that impedes DNA double-strand break end resection and CCF formation) for incorporation during CCF formation. This mechanism constitutes another mitochondria-to-nucleus retrograde signaling pathway that can activate the cGAS-STING pathway and develop the SASP (Vizioli et al., 2020). In senescent cells, downregulation of DNases (for example, DNase2 and TREX1) in the cytoplasm can also result in the accumulation of cytoplasmic DNA to aberrantly activate the cGAS-STING pathway (Takahashi et al., 2018).

Among the origins of cytoplasmic DNA are mammalian mitochondria, in which cytoplasmic DNA is created through the coordinated regulation of nuclear and mitochondrial genes. The mutation rate and progeronic potency of mtDNA are significantly higher than those of nuclear DNA, mostly owing to the ROS-rich mitochondrial microenvironment, the poor fidelity of mitochondrial DNA polymerase and the electron transport chain (ETC)-encoded ability (Li et al., 2019). Although only 13 of the 1500 proteins constituting mammalian mitochondria are encoded by mtDNA, they are all important components of the ETC that regulate oxidative phosphorylation (Taanman, 1999). Mutations in mtDNA with aging are positively linked with mitochondrial dysfunction, defects in ATP synthesis, and the exaggeration of ROS-induced oxidative stress (Niemann et al., 2017). It has also been proven that the declining mass of mtDNA in *Transcription Factor A Mitochondrial (TFAM)* knockout mice can induce systemic cytokine storms and multiorgan senescence featuring including muscular dystrophy, cardiovascular alterations and neurological disability through TNFα (Desdin-Mico et al., 2020). In the aging microenvironment, cell-free mtDNA acting as a DAMP or localized in EVs is released by senescent cells and can be recognized through the cGAS-STING pathway or pattern recognition receptors (PRRs), such as TRL9, to activate immune responses and increase the release of systemic inflammatory factors (Iske et al., 2020; Schwartz et al., 2021). In addition to mtDNA, DAMPs are dynamically generated, translocated and released into the extracellular milieu to increase senescence-related stressors, especially ROS, driving the initiation and development of aging and the SASP. With aging, the declining levels of intracellular DAMPs cause genomic instability, metabolic disorder, and chromatin modification, whereas increased levels of extracellular DAMPs can elicit immune and inflammatory responses via PRR recognition (Huang et al., 2015). For example, the nuclear high-mobility group box 1 (HMGB1) protein regulates DNA replication, transcription and repair, the loss of which can cause DNA damage and genome instability in the aging state (Jia et al., 2020). In contrast, extracellular HMGB1 is associated with TLR4-induced senescence-associated inflammation, which activates NF-κB signaling pathways and IL-6, IL-1 and TNFα secretion (Davalos et al., 2013).

It has also been reported that senescent $CD8^+$ T cells display the SASP but that it is governed by p38 MAPK signaling (Callender et al., 2018). This signaling pathway is dispensable along with the canonical DDR, and it can be directly mediated by mitochondrial dysfunction with decreased NAD+/NADH ratios. After activation of p38 MAPK, the levels of p53 and NF-κB also increase and then restrain and enhance the SASP, respectively (Freund et al., 2011; Wiley et al., 2016). In addition, the p38 MAPK signaling pathway can exert profound effects on oncogene-induced senescence models to increase the levels of secretory factors, an effect that is driven by high-mobility group A (HMGA)-mediated *NAMPT* expression and then NAD+-dependent AMPK derepression to enhance NF-κB activity (Nacarelli et al., 2019). Oncogenic Ras can also upregulate squamous cell carcinoma antigens 1 and 2 (SCCA1/2) through MAPK and the ETS transcription factor PEA3 to mediate NF-κB activation and inflammatory cytokine production (Catanzaro et al., 2014).

Furthermore, IL-1α and its receptors also reinforce NF-κB activity in an mTOR-dependent or S100A13-Cu^{2+}-dependent manner, even from the inflammasome, which accelerate the migration of IL-1α to the cell membrane and the binding of IL-1α with IL-1R in a juxtacrine fashion (Acosta et al., 2013; Ito et al., 2017; Laberge et al., 2015; Su et al., 2019b).

Regarding C/EBPβ, Notch signaling can regulate its temporal expression by regulating the level of TGF-β between an immunosuppressive profibrotic secretory state and a proinflammatory fibrolytic secretory state (Ito et al., 2017). In a RAS^{G12V}-induced senescence model, C/EBPβ can be rescued from 3′UTR regulation of protein activity (UPA) disruption and amplified by AMPK activation, leading to the SASP (Salotti & Johnson, 2019).

Accordingly, the SASP involves the release of a plethora of molecules to modify the aging microenvironment in a spatial and temporal manner via numerous transcription factors and signaling pathways. Expanding the SASP database and later improving the accuracy of SASP targeting through clinical trials may enable giant leaps to be made in aging research.

2.3 | Diversity of extracellular vesicles in cellular senescence

Initially, EVs were considered fragments and discarded units of dead and degenerated cells, but it was subsequently hypothesized that EVs are inevitably released during physiological and pathological processes (Misawa et al., 2020). EV classification is based on size, density, purification methods, formation progress, surface markers, and even release mechanisms. Although classification is still challenging, the International Society for Extracellular Vesicles (ISEV) holds that, based on the mechanisms of EV biogenesis, two identifiable subtypes of EVs have been largely confirmed: one from the plasma membrane (i.e., microvesicles, ectosomes, microparticles) and another from the endosomal system/multivesicular bodies (MVBs) (exosomes, which are vital) (Russell et al., 2019).

Vesicles directly formed by plasma membrane budding usually range from 100 to 1,000 nm in diameter (Moller & Lobb, 2020). They are rich in miRNAs, especially after oxidative stress, which enables them to be sorted into microvesicles by the hnRNPA2B1/caveolae-1 complex upon separate O-GlcNAcylation and phosphorylation (Tkach & Thery, 2016).

Exosomes are known for their nanoscale size and versatility, notably in cancer. Their biogenesis begins with endocytosis and invagination of the plasma membrane to form early-sorting endosomes (ESEs). After interaction with organelles and maturation into late-sorting endosomes (LSEs), they ultimately assemble multivesicular endosomes (MVEs). Intraluminal vesicles (ILVs) are then produced by secondary invagination of the endosomal limiting membrane in MVEs, a process mediated by the endosomal sorting complex required for transport (ESCRT) complex, sphingomyelinase, ceramide, RAB family members, and KIBRA (or their combinations) and are eventually released as exosomes. MVEs can merge with either lysosomes or autophagosomes to be degraded (Figure 3) (Kalluri & LeBleu, 2020; Moller & Lobb, 2020; Russell et al., 2019; Song et al., 2019; Wei et al., 2020a). Importantly, in either senescent or nonsenescent cells, inhibition of exosomal biogenesis through silencing of Alix or Rab27a, two key factors of EV production in the ESCRT-dependent pathway, can provoke ROS-induced DDR, cell cycle arrest and apoptosis owing to cytoplasmic DNA accumulation (Takahashi et al., 2017). This indicates that EV secretion pathways are essential to maintain cellular homeostasis.

Secreted EVs interact with or are internalized by recipient cells via classic receptor-ligand combination or endocytosis, including caveolae-dependent, clathrin-dependent and lipid raft-mediated endocytosis; receptor-mediated endocytosis; macropinocytosis; and phagocytosis (Feng et al., 2010; Gonda et al., 2019; Lia et al., 2020; Nanbo et al., 2013; Svensson et al., 2013; Tian et al., 2014). Specifically, oxidative stress-generated EVs can rapidly reduce transepithelial resistance in recipient cells through neuraminidase activity to enable their internalization (Nicholson et al., 2020).

EVs, as constituents of the SASP, show significant changes in senescent cells (Figure 3). These changes are primarily stimulated by irreparable DNA damage with aging that promotes sphingomyelinase and ceramide activity and increases Golgi dispersal and endoplasmic reticulum dilation accompanied by increasing inflammation and decreasing autophagy (Goulielmaki et al., 2020; Hitomi et al., 2020; Takahashi et al., 2017). In the DDR process, the production of EVs still relies on functional p53 and p65. Inhibition of p53 has been verified to reduce the secretion of $CD63^+$ EVs, which plays a pivotal role in senescence and apoptosis of adjacent cells (Tesei et al., 2021). It is possible that p53 activation enhances the expression of genes involved in endosome regulation and exosome generation during exposure to multiple stresses, such as CHMP4, caveolin-1 and nSMase2, which are important components of ESCRT-dependent and ceramide-dependent signaling pathways (Buratta et al., 2017; Yu et al., 2009). Inhibition of p65 also prevents age-induced inflammatory message transmission through EVs among mesenchymal stem cells, which reflects the importance of NF-κB in the SASP (Mato-Basalo et al., 2021).

EVs purified from PD donors are packed with low levels of mitochondrial markers (i.e., ATP5A, NDUFS3, and SDHB) related to systemic inflammation (i.e., CRP, MIP-1β, and TNF-α) (Picca et al., 2020). The existence of these mitochondrial markers as cargos suggest that mitochondrial dysfunction and impaired mitochondrial quality control (MQC) exist in PD in the presence of oxidative stress. The MQC system depends on the mitochondrial-lysosomal axis to achieve fusion and fission, which can dilute damaged mitochondria or clear them through mitophagy. If the system is compromised or overwhelmed, these damaged mitochondria enter the endolysosomal system to produce EVs with oxidized components requiring PINK1 and Parkin factors (Picca et al., 2019). These oxidized components, including proteins, lipids, nucleic acids and even ROS, can induce an inflammatory state in targeted cells (Borras et al., 2020). Furthermore, under oxidative stress, ROS in senescent cells can regulate cargo sorting into EVs by increasing the phosphorylation of proteins such as the receptor tyrosine kinase ephrin-A2 (EphA2) (Takasugi et al., 2017). Due to the vital role of mitochondrial dysfunction in aging, these mitochondrial components within EVs may become ideal molecular markers for individual ageotypes. However, in mitochondria-free red blood cells (RBCs), aging EVs are released through membrane shedding by haemoglobin (Hb)-generated ROS (Fibach, 2021). In addition, the lipid peroxidation product 4-hydroxynonenal (HNE) can also elevate the production and internalization of EVs containing cytotoxic α-synuclein to propagate pathology transneuronally in Parkinson's disease (Zhang et al., 2018b). Although several senescence-associated pathways

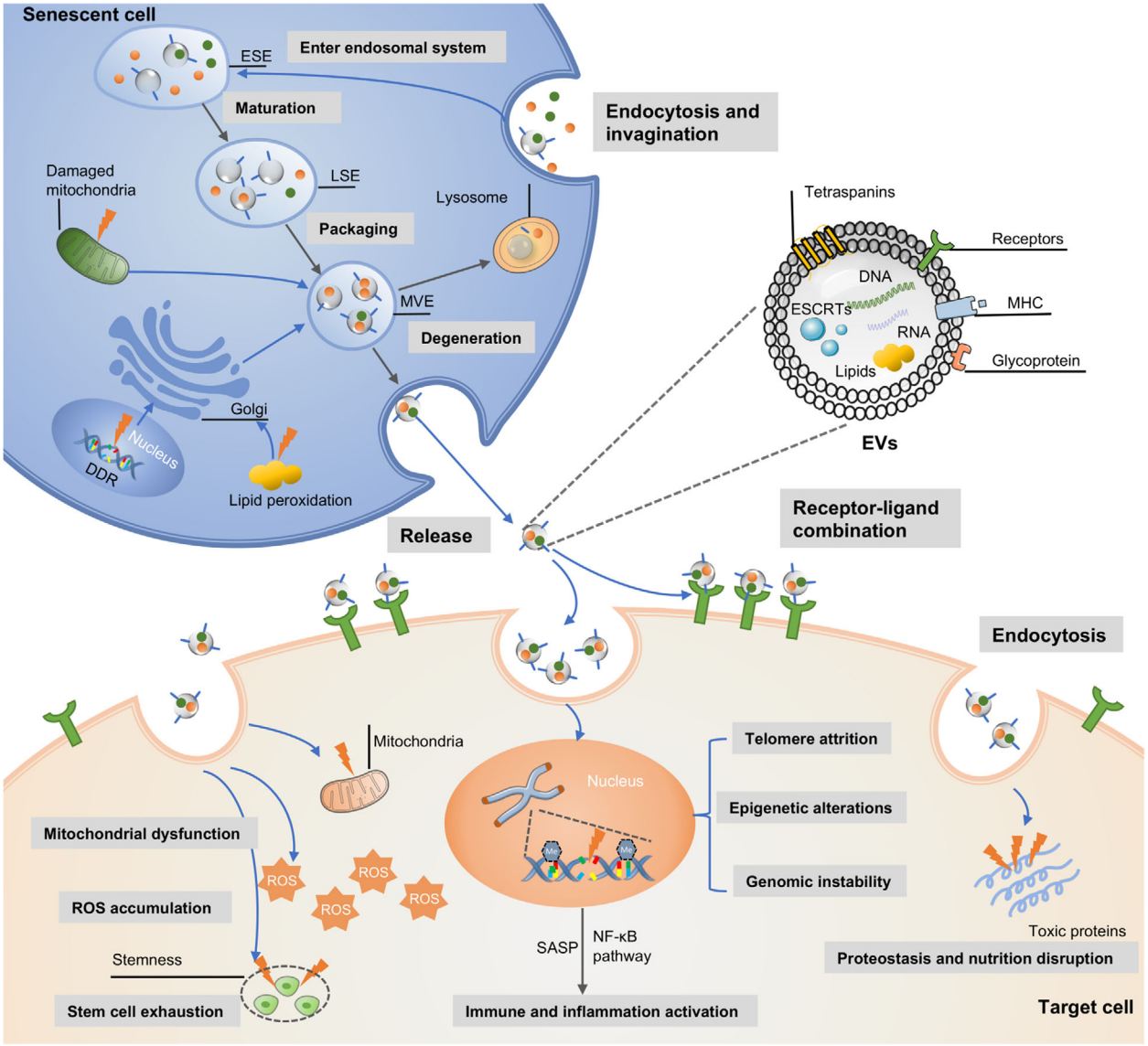

FIGURE 3 Mechanisms of EVs in the aging microenvironment. Senescence-associated EVs are produced from the plasma membrane and the endosomal system, and their levels can be increased due to DNA damage, mitochondrial damage and lipid peroxidation with age. These EVs contain typical markers (tetraspanins, ESCRT proteins, MHC, etc.) and specific proteins, glycoproteins, nucleic acids, and lipids (such as ceramide and sphingomyelin). They carry destructive signals and shuttle from senescent cells to target cells, which causes aging transmission. Target cells internalize these EVs through receptor-ligand combinations or endocytosis. Senescence-associated EVs can alter and induce senescence in target cells through immune and inflammatory activation, genomic instability, telomere attrition, epigenetic alterations, mitochondrial and lysosomal system dysfunction, ROS accumulation, loss of proteostasis and nutrition, and stem cell exhaustion. Here, we only present the route of exosome production. Early-sorting endosomes, ESEs; late-sorting endosomes, LSEs; multivesicular endosomes, MVEs; and intraluminal vesicles, ILVs

have been confirmed to participate in EV secretion, the detailed molecular mechanism by which EV biogenesis is regulated with aging remains largely unexplored.

The particle number and size of EVs have been tracked and compared under senescent and nonsenescent conditions in several experiments. However, the results have been controversial, possibly due to different cell derivations, senescence triggers, and scale ranges. Even though one report has supported categorization based on a concomitant increase in the number of relatively small EVs (Basisty et al., 2020; Chanda et al., 2019; Lei et al., 2017), another report has argued for categorization of EVs of similar number and size (Alberro et al., 2016; Fafian-Labora et al., 2020a). Notably, the heterogeneity of EV cargos determines the diversity of EV functions in the aging microenvironment. EVs may contain typical markers (tetraspanins, ESCRT proteins, MHC, etc.) and specific proteins, glycoproteins, noncoding RNAs, and lipids (such as ceramide and sphingomyelin) during aging (Khayrullin et al., 2019; Pienimaeki-Roemer et al., 2017; Sagini et al., 2018; Willis et al., 2020). The cargos can be expressed either ubiquitously or in a tissue-specific manner and used as biomarkers to capture aging complexity. We describe the diverse cargos and multiple functions of EVs in the aging microenvironment and age-related diseases in detail below.

3 | MECHANISM OF AGING TRANSMISSION VIA EXTRACELLULAR VESICLES

With aging or senescence-associated stress, the dynamic cargos of EVs reach their targets through paracrine or endocrine patterns and exhibit destructive or supportive behaviours that affect aging signal transmission. This transmission refers to the passing of information via cell-to-cell or organ-to-organ communication through EV trafficking (Figure 3). Borghesan et al. confirmed that IFITM3, a protein messenger linked with the IFN pathway, was selectively encapsulated in small EVs during oncogene-induced senescence (OIS). IFITM3 is critical for early genotoxic damage and paracrine senescence in neighbouring fibroblasts (Borghesan et al., 2019). Moreover, shuttling of EVs between the liver and vasculature in a non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) model has revealed the distant interorgan communication of EVs. Steatotic hepatocyte-derived EVs containing miR-1 suppress KLF4 expression but activate the NF-κB pathway to facilitate endothelial inflammation and senescence and participate in the development of atherosclerosis (Jiang et al., 2020). In these ways, transmission through EVs from senescent cells has negative impacts on the aging microenvironment, promoting chronic low-grade inflammation and cellular senescence in recipient cells and organs and progressively inducing senescence-associated diseases.

EVs isolated from senescent cells can transform recipient cells' immune state to mediate cellular viability through information delivery (Figure 3). When treated with oxidative agents, retinal pigment epithelial cells, a type of phagocytic cell, can produce more CD36-associated microparticles, impeding phagocytosis and diminishing the viability of neighbourhood cells, as indicated by increased SA-β-gal staining and p21 and p15 expression (Yang et al., 2020). A cell-free form of telomeric repeat-containing RNA (cfTERRA) is abundant in EVs and is related to telomere dysfunction and DNA damage of parent cells. It is regarded as a telomere-associated molecular pattern (TAMP) or DAMP and is detected in the blood plasma of control subjects and cancer patients, directly revealing that exosomal cfTERRA is a potential marker for telomere metabolism in senescence (Azzalin & Lingner, 2015; Cusanelli et al., 2013; Porro et al., 2014). It can also induce the transport of alarmin to elicit a recipient inflammatory response (Wang & Lieberman, 2016). Incubation of peripheral blood mononuclear cells with exosomes containing cfTERRA stimulates innate immune signaling and upregulates inflammatory cytokines, such as TNFα, IL-6, and CXCL10 (Wang et al., 2015). In addition to cfTERRA, other exosomal DAMPs modulate innate immunity and cell conditions in the aging microenvironment. $CD63^+$ EVs from irradiated cancer cells loaded with DNA:RNA hybrids and LINE 1 retrotransposons trigger the senescence of adjacent cells and induce innate immune signaling to polarize M1 macrophages (Tesei et al., 2021). EVs from senescent oral keratinocytes can carry mtDNA and nuclear DNA to activate the cGAS-STING pathway and interferon expression in monocytes (Schwartz et al., 2021). Hence, these DAMPs in EVs consequently contribute to chronic inflammation and age-related diseases through the innate immune signaling pathway, which can be considered a positive feedback loop of SASP modulation.

In genotoxic aging models with either irradiated breast cancer cells or irradiated mice, EVs can directly induce telomere attrition and DDR in recipients, causing genomic instability and aging. However, this effect is reduced by RNase treatment or thermal denaturation by boiling, which suggests that protein- and RNA-containing EVs indeed have effects on telomere shortening and DNA damage (Al-Mayah et al., 2017; Hargitai et al., 2021). In addition, EVs can mediate genomic instability through senescence-associated genome-wide hypomethylation to spread senescence characteristics to neighbouring cells. In a model consisting of replicative senescent human umbilical vein endothelial cells (HUVECs), EVs carried miR-21-5p, and miR-217 downregulated DNA methyltransferase 1 (DNMT1) and Sirtuin 1 (SIRT1) expression in young cells, leading to high levels of SASP molecules and cell cycle inhibitors (Mensa et al., 2020). In nature, DNMT1 cooperates with SIRT1 (a well-known longevity-related gene) to maintain genetic methylation patterns in order to ensure genome integrity through mitosis (Lee et al., 2019; Zheng et al., 2015). Therefore, EVs transmit aging signals at the genetic and epigenetic levels (Figure 3).

Cargos and their corresponding EVs can co-destroy energetic and proteinic networks to upset the intracellular balance of the targeted cells, promoting aging (Figure 3). In a model of recipient mitochondrial dysfunction, lung fibroblast-derived EVs from idiopathic pulmonary fibrosis (IPF) patients carried elevated levels of miR-23b-3p and miR-494-3p to suppress SIRT3 expression. These EVs are critical for increasing mitochondrial ROS, causing damage, and inducing subsequent lung epithelial cell senescence, which verifies that EVs can destroy the energetic networks of target cells to induce senescence (Kadota et al., 2020). With aging, overload of the local lysosomal system can lead to a decrease in cellular degradation capacity, especially for some negligibly digested proteins, such as α-synuclein, in PD. In transgenic *Caenorhabditis elegans*, these misfolded proteins are transported into EVs migrating from neurons or from muscle to the hypodermis, which disrupts intracellular protein homeostasis of distant cells after rupture (Sandhof et al., 2020). EVs can also abrogate proteostasis through autophagic disruption of recipient cells. In a study using *Caenorhabditis elegans* to study EV trafficking, miR-83-enriched EVs, which drift from the intestine to other tissues in pseudocoelomic fluids, was found to limit the expression of CUP-5 (an autophagic regulator), obviously reducing autophagy in distinct tissues and causing protein aggregation and senescent phenotype acquisition (Zhou et al., 2019). In addition, EVs can modulate targets' anabolic activities through accumulation of the very-long-chain ceramide C24:1 (Khayrullin et al., 2019), which negatively affects amino acid transporters to starve cells to death (Guenther et al., 2008).

Stem cell exhaustion is another pivotal factor playing a role in EV-linked aging transmission (Figure 3). Mesenchymal stem cells (MSCs) from bone marrow are among the widely reported stem cells affected by EVs. Incubation of young MSCs with old MSC-derived EVs stimulates the mTOR pathway, promotes aging marker expression and reduces pluripotency marker levels

(Fafian-Labora et al., 2020a). Incubation of bone marrow stem cells (BMSCs) with EVs from aged bone marrow interstitial fluids consistently induces stem cell senescence and impedes osteogenic differentiation via the miR-183 cluster (Davis et al., 2017). Moreover, mouse myoblasts and primary human myotubes treated with hydrogen peroxide generate miR-34a-carrying circulating EVs to suppress SIRT1 and induce BMSC senescence and death (Fulzele et al., 2019). Therefore, these shuttled EVs seem to dictate stem cell differentiation, maturation, cessation, and even expiration. However, more convincing in vivo data to verify the impacts of EVs on stem cells are still lacking.

In conclusion, EVs and their cargos play important roles in transmitting aging signals through intersecting networks, partially inducing immune and inflammatory activation, genomic instability, telomere attrition, epigenetic alterations, mitochondrial and lysosomal system dysfunction, ROS accumulation, loss of proteostasis and nutrition, and stem cell exhaustion in targeted cells (Figure 3). All these mechanisms are also prime aging hallmarks of parent cells, but the transmission through EVs is not just a senescent copy. It is a fine-tuned informative delivery and systemic response. Loss of balance may contribute to senescence-associated diseases. However, more experiments should be performed to strengthen the evidence, and more attention should be given to the molecular function of EVs in aging transmission and pathology.

4 | IMPACTS OF EXTRACELLULAR VESICLES ON AGE-RELATED DISEASES

Aging is one of the risk factors for chronic diseases and dysfunctional pathologies. In age-related diseases, senescent cells exert central effector functions to transmit SASP factors to neighbouring cells and tissues, among which different EVs could be representative. However, senescent cells are highly heterogeneous and form multiple colonies; thus, senescent cell-secreted EVs have different cellular origins and tissue specificities (Table 2).

4.1 | Bone diseases

Bone is a dynamic tissue regulated by life-long formation and resorption that requires stem cells, osteoclasts, and osteoblasts to cooperate precisely. Once bone equilibrium is disrupted, redundant bone resorption and reduced bone formation can cause osteopenia and osteoporosis (Li et al., 2016). Bone loss is a more typical feature of elderly individuals than young individuals. This pattern of disease indicates that impaired intercellular contacts strongly collaborate with the senescence process in bone loss.

Mesenchymal stem cells (MSCs) in bone marrow are multipotent stem cells based on their ability to differentiate into chondrocytes, osteoblasts, adipocytes, and stromal cells to maintain osseous tissue homeostasis and effective performance in bone remodelling and regeneration (Wolock et al., 2019). However, aged BMSCs show a decline in proliferation rate, loss of stemness and viability, decreased osteogenic and chondrogenic differentiation, and increased adipogenesis (Baker et al., 2015). With aging, BMSCs are susceptible to excessive ROS deposition and energy disorder on the path to exhaustion and anergy (Denu & Hematti, 2016). The two main intrinsic factors (ROS deposition and energy disorder) may be effectively controlled by depletion of inositol hexakisphosphate kinase 1 (Ip6k1), enhancing BMSC growth and survival. Ip6k1 is usually described as a regulator of fat accumulation through AMPK-mediated adipose energy metabolism (Boregowda et al., 2017; Zhu et al., 2016).

As mentioned previously, extrinsic factors, such as EVs, also generate harmful impacts on the aging process by transmitting miRNA signals. In osteoporosis, it has been experimentally proven that the crosstalk between BMSCs and osteoclasts relies on EV conveyance of information in bone marrow. MiR-31a-5p derived from aged BMSCs mechanically binds to the 3′UTR of the RhoA gene to inhibit osteoclast cytoskeletal organization in a paracrine manner while directly attaching to the SATB2 and IE2F2 genes in an autocrine manner to decrease self-stemness and osteogenic differentiation (Xu et al., 2018b; Zhou et al., 2016). Osteoclasts purified from ovariectomized mice transport EVs containing miR-214-3p to cocultured osteoblasts, ultimately inducing osteoclastogenesis through the PI3K/AKT pathway (Li et al., 2016). Other cells, such as senescent endothelial cells, promote osteoporosis development via miR-31 transmission to MSCs, inhibiting their capacity for differentiation (Weilner et al., 2016). Notably, exosome functions in the serum of osteoporosis and osteopenia patients are contradictory. The latter demonstrate a compensatory elevation in bone formation. These contradictory findings suggest that greater attention should be given to the ageotypes of elderly patients when considering the use of EVs as diagnostic biomarkers.

Osteoarthritis (OA) is another common age-associated bone disease characterized by distinct chondrocyte catabolism and death, cartilage matrix loss, and an inflammatory state due to oxidative stress and the SASP mode (Loeser et al., 2016). The contents of EVs in synovial fluids differ significantly under osteoarthritis conditions and by sex. Patients with severe OA tend to show stimulated release of proinflammatory factors from M1 macrophages (Domenis et al., 2017; Kolhe et al., 2017). In females, EVs are involved in the ovarian steroidogenesis signaling pathway and metabolic process (Kolhe et al., 2017). In OA-affected joints, chondrocytes and immune cells also deliver pathogenic signals reciprocally via EV shuttling (Zhou et al., 2020). Taking miR-449a-5p as an example, senescent OA chondrocytes produce miR-449a-5p vesicles to repress ATG4B gene expression in macrophages and then inhibit their autophagy. Subsequently, ROS deposition leads to inflammasome activation and IL-1β release

TABLE 2 Roles of extracellular vesicles in age-related diseases

Disease	Senescent cell sources	Cargo(s)	Mechanism(s) and Effect(s)	Ref.
Musculoskeletal diseases				
Osteopenia and osteoporosis	Osteoclasts	miR-214-3p	Promoted osteoclastogenesis and reduced bone formation through PI3K/AKT pathway	(Li et al., 2016)
	BMSCs	miR-31a-5p	Increased adipogenesis and aging phenotypes; Promoted osteoclastogenesis and bone resorption	(Xu et al., 2018b)
	HUVECs	miR-31	Inhibited osteogenic differentiation of mesenchymal stem cells via Frizzled-3 restraint	(Weilner et al., 2016)
Osteoarthritis	Chondrocytes	/	Reduced cartilage production and increased the inflammatory state and pain	(Jeon et al., 2019)
	Degraded cartilage	miR-449a-5p	Repressed the expression of the ATG4B gene, leading to inhibition of autophagy; Increased ROS accumulation and inflammasome and IL-1β activation	(Ni et al., 2019)
Neurodegeneration				
Alzheimer's disease	Astrocytes and neurons	/	Induced MAC formation and neurotoxicity on recipient neurons	(Nogueras-Ortiz et al., 2020)
	Microglia	Tau protein	Microglia phagocytosed Tau protein and facilitated tau propagation to neurons	(Asai et al., 2015)
	CSF	BIN1 protein	Reduced microglial Tau clearance, increased Tau release and propagation	(Crotti et al., 2019)
	SHswe cells	miRNAs	Induced proinflammatory factor expression and aging in microglia	(Fernandes et al., 2018)
	Neuroblastoma cells; AD CSF	Tau protein	Facilitated Tau protein transmission depending on synaptic connectivit; Promoted Tau aggregation in secondary neurons and microglia	(Wang et al., 2017b)
Parkinson's disease	Neuroblastoma cells	Gangliosides	Catalyzed the conversion of α-synuclein monomeric pattern to fibrillar aggregates	(Grey et al., 2015)
	HEK293 cells	α-Synuclein	Facilitated α-synuclein uptake and dysregulation in monocytes and microglia	(Grozdanov et al., 2019)
	LPS-treated erythrocytes	α-Synuclein	Traversed the blood-brain barrier and upregulated microglial inflammatory responses; Promoted the process of synucleinopathy pathogenesis	(Matsumoto et al., 2017)
	Microglia	α-Synuclein	Dysregulated autophagy of the patient cells and accelerated secretion of α-synuclein	(Xia et al., 2019)
Cardiovascular diseases				
Vascular calcification	ECs and plasma	Annexins, Ca^{2+}	Promoted calcification in vascular smooth muscle cells	(Alique et al., 2017)
Atherosclerosis	TNF-α treated ECs	/	Disseminated SASP molecules and promoted the monocyte inflammatory mode; Promoted atherosclerotic plaque formation	(Hosseinkhani et al., 2018)
	Atherogenic macrophages	miR-146a	Repressed the expression of IGF2BP1 and the HuR gene; Decreased migration and promoted entrapment of macrophages	(Nguyen et al., 2018)
Cardiac hypertrophy	Cardiac fibroblasts	miR-21-3p	Mediated cardiac hypertrophy and remodeling through downregulation of the genes SORBS2 and PDLIM5	(Bang et al., 2014)
	Angiotensin-II treated fibroblasts	/	Upregulated the expression of renin, angiotensinogen, Ang II receptor, and Ang II in cardiomyocytes via the MAPK and AKT pathways	(Lyu et al., 2015)
	Fibroblasts	miR-27a	Induced ROS with hypertrophic gene expression and suppressed PDLIM5 gene expression	(Tian et al., 2020)
Heart failure	Fibroblasts	miR-146a	Suppressed SUMO1 expression, SERCA2a SUMOylation and cardiac contractility	(Oh et al., 2018a)

(Continues)

TABLE 2 (Continued)

Disease	Senescent cell sources	Cargo(s)	Mechanism(s) and Effect(s)	Ref.
Cardiac fibrosis	Hypertrophied myocytes	Hsp90	Activated STAT-3 signaling of cardiac fibroblasts and enhanced excess collagen synthesis	(Datta et al., 2017)
	Myocytes under hypoxia	LncRNA Neat1	Mediated fibroblastic cell survival, fibrosis, proliferation and migration via the AKT pathway	(Kenneweg et al., 2019)
Type 2 diabetes mellitus	Diabetic plasma	inflammatory proteins and VEGF-A	Increased lamellipodia formation and migration in endothelial cells; Increased the potential for peripheral vascular diseases	(Wu et al., 2020)
	Erythrocytes	/	Altered the expression of genes related to cell survival, oxidative stress and immune function in leukocytes; increased inflammatory cytokine levels	(Freeman et al., 2018)
	BMSCs	miR-29b-3p	Inhibited the insulin signaling pathway; Exacerbated insulin resistance of young BMSCs through SIRT1 dysfunction	(Su et al., 2019a)
	Adipose tissue macrophages	miR-155	Reduced insulin sensitivity of insulin target cells through PPARγ suppression; Induced insulin resistance and glucose intolerance	(Ying et al., 2017)
	Adipocytes	miR-27a	Decreased IRS-1, GLUT4 and AKT in insulin target cells through PPARγ inhibition; Impaired glucose consumption and insulin sensitivity of skeletal muscle cells	(Yu et al., 2018)
	High glucose-treated ECs	Notch 3	Increased the expression of osteocalcin, p21 and SA-β-gal in vascular smooth muscle cells; Increased VSMC calcification, aging and the potential for cardiovascular diseases	(Lin et al., 2019)
	Albumin-treated renal tubular epithelial cells	miR-4756	Increased epithelial-to-mesenchymal transition and endoplasmic reticulum stress in tubular epithelial cells through Sestrin2 inhibition; Accelerated diabetic kidney disease progression	(Jia et al., 2019b)
Reproductive diseases				
Ovarian aging	Follicular fluid of elderly women	miR-21-5p; miR-134; miR-190b	Regulated heparan-sulfate proteoglycan expression and deregulated follicle morphogenesi; Increased apoptosis of human granulosa cells through regulation of the p53 pathway, bcl2, and IKBKG	(Diez-Fraile et al., 2014)
	Follicular fluid of elderly women	miR-16; miR-214; miR-372	Increased apoptosis and cellular senescence though activation of the p53 pathway; Increased EV production through feedback	(Battaglia et al., 2020)
	Follicular fluid of aged mares	miR-181a	Decreased proliferating cell nuclear antigen (PCNA) levels of granulosa cells; Suppressed the TGF-β pathway and impaired oocyte maturation	(da Silveira et al., 2015)
	Plasma in POF patients	miR-23a	Increased apoptosis of granulosa cells through XIAP inhibition and caspase-3 activation	(Yang et al., 2012)
Uterine aging	Endometrial stromal cells	PAI-1	Transmitted senescence patterns in 2D and 3D senescent and young ESC coculture systems; Perhaps dysregulated endometrial function and impaired embryo implantation	(Griukova et al., 2019)

(Ni et al., 2019). Therefore, eliminating senescent cells or interfering with EV contents may be beneficial to osteoporosis and osteoarthritis.

4.2 | Neurodegeneration

As the frequencies of neurodegenerative diseases exponentially increase with age, postmitotic cells in the brain show extreme sensitivity to systemic and cellular senescence. Alzheimer's disease (AD) and Parkinson's disease (PD) are the most extensively studied neuronal senescence-associated conditions to date, and both are mainly attributed to insoluble misfolded protein aggregation in specific regions of the brain. These neuropathic proteins are closely linked with senescence-associated autophagy and lysosomal dysfunction, which lead to their stable presence in specific EVs (Chung et al., 2019; Kapogiannis et al., 2019;

Lamontagne-Proulx et al., 2019; Minakaki et al., 2018). Additionally, evidence has shown that injection of EVs isolated from AD patients into the hippocampi of wild-type mice is neurotoxic in vivo, leading to increased tau phosphorylation (Aulston et al., 2019). After such EVs are intravenously injected from the serum of PD patients into mice, protein aggregation, dopamine neuron degeneration, microglial activation, and mouse movement defects are also observed (Han et al., 2019a). This directly indicates that EVs packaged with a pathogenic pattern of molecules may play vital roles in neural aging phenotype transmission.

To precisely evaluate and diagnose age-associated neurodegeneration, secreted EVs in various body fluids may be the optimal biomarkers. Taking advantage of blood-brain barrier permeability to EVs, brain-derived exosomes can be detected in the peripheral circulation. Neuron-derived EVs in serum, sorted by L1CAM and neural cell adhesion molecule expression, were found to exhibit elevated phosphorylated tau-181 and 231, Aβ42 and insulin receptor substrate 1 levels in an AD group (Kapogiannis et al., 2019). Compared to exosome-unbound Aβ, exosome-bound Aβ can better reflect amyloid plaques of the brain and be used to differentiate various clinical groups via amplification techniques (Lim et al., 2019). The levels of advanced glycation end products N-(1-carboxymethyl)-L-lysine (CML) in EVs present dynamic changes in early and moderate AD (Haddad et al., 2019). However, there is little correlation in the miRNA contents of EVs in brain and blood samples obtained from AD subjects (Cheng et al., 2020), while similar levels of exosomal Aβ42 and tau in CSF and blood have been verified (Jia et al., 2019a). A novel tau fragment in AD, N-224 tau, can be packaged in neuron-specific EVs and secreted in CSF to upregulate cognitive decline (Cicognola et al., 2019). In PD, in addition to α-synuclein detected in CSF, numerous noncoding RNAs are significantly upregulated in EVs, including miR-153, miR-409-3p, and let-7 g-3p (Gui et al., 2015; Stuendl et al., 2016). Interestingly, the α-synuclein oligomer and the total ratio of α-synuclein in salivary EVs can be noninvasively assessed in PD patients, and α-synuclein levels increase with disease severity (Cao et al., 2019). Consequently, it is valuable to classify specific EVs in biofluids as ageotypes and obtain profiles of AD and PD patients for early and safe diagnosis.

In particular, EVs can contribute to the development and aggravation of these neurodegenerative diseases through the intercellular transmission of misfolded proteins or other pathological molecules. In AD, EVs with tau protein are released by primarily affected neurons after depolarization and internalized by the next neurons through synaptic transmission to cause protein folding errors in normal cells (Wang et al., 2017b). SHswe cells (a neuroblastoma cell line transfected with the Swedish mutant of amyloid precursor protein) are capable of stimulating the microglial immune response and increasing senescence hallmark activation through EV-containing miRNA transmission between neurons and microglia (Fernandes et al., 2018). Microglia, which are tissue-resident macrophages, not only facilitate tau release in EVs but also facilitate neuroinflammation development via EVs (Asai et al., 2015). With age, the accumulation of internal antigen-antibody compounds and misfolded proteins stimulates microglia to overexpress several complement components involved in the complement cascade and maladaptive neuroinflammatory effects (Spani et al., 2015). High complement levels can also be found in astrocytic EVs and neuronal EVs (Goetzl et al., 2018). These inflammatory EVs cause cellular membrane disruption and neurite density reduction in rat cortical neurons by activating membrane attack complex (MAC) formation (Nogueras-Ortiz et al., 2020). Age-associated chronic inflammatory states are similarly evident during PD progression. α-Synuclein in EVs, whether from the brain or the periphery, can incessantly evoke innate immune responses and interfere with the clearance system, resulting in synucleinopathy pathogenesis (Grozdanov et al., 2019; Matsumoto et al., 2017; Xia et al., 2019). In a sense, pathological pattern transmission via EVs is equivalent to aging phenotype transmission, as both involve crosstalk of diverse cells in the brain microenvironment and induce systemic immune responses to low-grade inflammation.

4.3 | Cardiovascular diseases

Cardiovascular diseases are the most prevalent types of age-associated conditions and manifest as structural, cellular, molecular, and functional changes in the heart that occur with aging. Numerous circulating progeronic and antigeronic factors from different systems orchestrate the aging process simultaneously in the cardiovascular system. They can cause vascular aging and subsequently impair angiogenesis and induce chronic inflammation, pathological remodelling, atherogenesis, and myocardial injury (Fajemiroye et al., 2018; Ungvari et al., 2018). In the endocrine system, glucose stimulation aggravates endothelial dysfunction and increases NADP (nicotinamide adenine dinucleotide phosphate) oxidase activity and ROS levels, producing more senescent endothelial EVs that mediate cardiovascular diseases in diabetes (Carracedo et al., 2019). Other factors, such as proinflammatory cytokines (IL-1, TNF-α, and INF-γ), coagulant factors (thrombin and PAI-1), hypoxia, and low shear stress, can also stimulate endothelial cells to release progeronic EVs (Hromada et al., 2017).

Vascular calcification, a risk factor for atherosclerosis and myocardial injury, is markedly induced by age-related EVs. EVs extracted from replicative senescent endothelial cells (ECs) and the plasma of elderly patients contained increased levels of annexins, BMP2 (a bone morphogenic protein) and Ca^{2+}, which induce the initiation and propagation of calcification in human aortic smooth muscle cells (Alique et al., 2017; Pescatore et al., 2019). The interaction between monocytes and ECs also plays a crucial role in inflammatory atherosclerosis. TNF-α-induced ECs can produce more EVs containing chemotactic mediators, such as ICAM-1, CCL-2, CXCL-10, and CCL-5, which are taken up by monocytes to promote the expression of their inflammatory markers (IL-6 and IL-8) and their adhesion and migration (Hosseinkhani et al., 2018). On the other hand, activated monocytes

constitute mitochondria-rich EVs and initiate type I INF and TNF signaling pathways in ECs, leading to chronic inflammation (Puhm et al., 2019). In addition, atherogenic macrophages can decrease migration and promote the entrapment of self- or naïve macrophages through inhibition of IGF2BP1 and HuR expression by miR-146a-associated EVs (Nguyen et al., 2018). Elucidating the intercellular trajectory, mediators, and cargos of EVs will aid in attenuation of vascular calcification and atherosclerosis.

In acute myocardial infarction, EVs are transiently increased and mainly originate in myocytes and ECs. Monocytes can readily take up these EVs to regulate inflammatory responses in the local region (Loyer et al., 2018). The evidence supports the idea that the ischemic myocardium selectively releases EVs from leukocytes, ECs and myocytes in vivo. Concomitantly, EVs from myocytes upregulate MMP3 and other genes associated with apoptosis and senescence in ECs (Rodriguez et al., 2018). These age-affected myocardial injured tissues undergo cardiomyocyte hypertrophy, apoptosis, cardiac fibrosis, and angiogenesis in the interplay among myocytes, ECs, fibroblasts, and immune cells. Between myocytes and fibroblasts, EVs from fibroblasts join in the pathological process of cardiac hypertrophy and heart failure. MiR-21-3p and miR-27a have been proven to suppress the PDLIM5 gene of myocytes and decrease cardiac contractility, which mainly mediates cardiomyocyte hypertrophy and remodelling, ultimately leading to heart failure (Bang et al., 2014; Tian et al., 2020). Together, the evidence indicates that myocytes exert a profound influence on cardiac fibrosis through EV trafficking. Deposition of extracellular matrix is markedly reduced in fibroblasts conditioned with supernatant from Hsp90-inhibited hypertrophied myocytes. In fibroblasts, Hsp90 inhibition decreases IL-6 levels and STAT-3 activation to ameliorate cardiac fibrosis (Datta et al., 2017). Interestingly, EVs from aged mouse serum contain reduced levels of Hsp70. However, the inhibition of Hsp70 promotes fibroblast proliferation and fibroblast-to-myofibroblast differentiation (Yang et al., 2019). Fortunately, EVs from stem cells usually display positive phenotypes in cardiac trauma repair to ameliorate postinfarction remodelling and attenuate ischemic progression. We discuss the functions of EVs from stem cells in further detail below. Blockade of pathological EV communication among these cells or utilization of stem cell therapy are methods that can be used to attenuate myocardial injury and ischemic diseases.

4.4 | Type 2 diabetes mellitus (T2DM)

It has been confirmed that aging greatly increases susceptibility to chronic and refractory age-related diseases. In turn, age-related diseases, in a mechanism similar to positive feedback, exacerbate the aging microenvironment and accelerate the aging process. In particular, a diabetic microenvironment is replete with factors leading to senescence and hyperglycaemia, both of which collaborate to induce long-standing diabetes mellitus and severe complications. In the endocrine system, message delivery via EVs is essential for realization of aging transmission. In a diabetic microenvironment, EVs from MSCs are aging factor carriers that induce renal tubular cell senescence, fibrosis and dysfunction (Li et al., 2020c). These outcomes indicate that stem cells problems should be monitored when they are utilized in tissue repair of T2DM patients.

The increased EVs from the circulation and cells induce diabetic senescence and organ injury, subsequently causing systemic inflammation and insulin resistance. Evidence has shown that mouse islets under attack from mixed cytokines and streptozotocin can produce dramatically elevated amounts of injury-related exosomal miR-375-3p in serum prior to the development of blood glucose and insulin disorders (Fu et al., 2018). In addition, $CD31^{+}$/annexin V-labelled EVs from damaged endothelial cells show higher sensitivity in T2DM than in metabolic syndrome, which leads to immune cytokine overproduction and vascular aging (Berezin et al., 2015). Research on EVs obtained from T2DM plasma, mainly erythrocyte-derived EVs, has revealed the immune function of these EVs, which are internalized by circulating leukocytes; this internalization alters their gene expression related to cell survival, oxidative stress and cytokine release (Freeman et al., 2018).

Bone can be regarded as another endocrine tissue that regulates systemic hormone metabolism. BMSCs from aged mice target SIRT1 in adipocytes, myocytes and hepatocytes through exosomal miR-29b-3p to significantly induce age-related insulin resistance in vitro and increase blood glucose in vivo (Su, Xiao et al., 2019). Moreover, EVs from obese tissue engage in age-related DM. Excessive calorie intake in mice can induce cellular senescence in adipose tissue through accumulation of ROS and activation of p53, which have been proven to play roles in insulin resistance (Minamino et al., 2009). Thereinto, exosomal miR-27a in adipocytes has been verified to participate in the insulin resistance of muscle cells through repression of downstream PPARγ (Yu et al., 2018). In addition to adipocytes, macrophages from adipose tissue can release EVs to modulate cellular insulin action via the same target gene (Ying et al., 2017). These findings suggest that controlling energy intake not only maintains glucose homeostasis but also ameliorates senescence.

However, the true risk of diabetes involves its complications, especially microvascular and macrovascular diseases. Long-term exposure to high glucose causes endothelial cells to transfer exosomal Notch3 to vascular smooth muscle cells, consequently increasing senescence and osteocalcin expression to induce vascular calcification and aging (Lin et al., 2019). Additionally, renal tubular epithelial cells are injured by albumin in diabetic macroalbuminuria. Exosomal miR-4756 accelerates the aging and injury of these cells by suppressing Sestrin2 to induce endoplasmic reticulum stress and the epithelial-to-mesenchymal transition (Jia et al., 2019b). Despite the multiorgan hazards and refractoriness of T2DM, metformin therapy has been confirmed as a jack-of-all-trades drug to achieve antiaging, antidiabetes, and antiobesity effects (Cabreiro et al., 2013; Onken & Driscoll, 2010). A spectrum of circulating miRNAs in EVs in T2DM subjects treated with metformin has been found to be surprisingly similar to

that of healthy controls (Ghai et al., 2019). However, caution should be exercised when considering the use of metformin as an antiaging agent for treating healthy elderly patients. A recent study warns that it will enhance mitochondrial dysfunction and metabolic disturbance in late life, shortening lifespan (Espada et al., 2020).

4.5 | Reproductive diseases

Reproductive aging, including ovarian aging and uterine aging, is the earliest type of system senescence in women, as the ovaries exhibit gradual declines in follicle quantity and quality from birth onwards. Detrimental effects of reproductive aging mainly affect oocyte quality and reserves, female hormonal balance, energy metabolism, and pathological changes. Evidence has highlighted that reduced fertilization and implantation and increased miscarriage occur in women of child-bearing age with ovarian aging. A single-cell transcriptomic analysis of the ovaries in young and old nonhuman primates demonstrated that the expression of genes involved in oxidative phosphorylation and oxidoreductase activity pathways was reduced in aging oocytes and granulosa cells, contributing to oxidative damage and apoptosis (Wang et al., 2020). Another single-cell transcriptomic analysis in the ovaries of aged mice after supplementation with nicotinamide mononucleotide (NMN), an NAD^+ intermediate, showed that mitochondrial function was significantly restored with ROS elimination after NMN supplementation (Miao et al., 2020). Both studies showed that mitochondrial dysfunction had adverse effects on oocyte function with aging.

In addition to mitochondrial dysfunction in the ovaries, apoptosis causes infertility and ovarian aging through senescence transmission by EVs. Downregulation of X-linked inhibitor of apoptosis protein (XIAP) has been found to occur in aged granulosa cells via miR-23a, which activates caspase-3 and increases apoptosis of granulosa cells (Yang et al., 2012). In follicular fluid obtained from older women, exosomal miR-134 has also been found to be potentially involved in an apoptotic pathway upon targeting of Bcl2 and IKBKG (Diez-Fraile et al., 2014).

In endometrial stromal cells, EVs also transmit a senescent phenotype in 2D and 3D cell coculture systems using aged and young cells, which dysregulates uterine function during pregnancy and impairs embryo implantation (Griukova et al., 2019). However, there is little evidence to confirm that senescent EVs are strongly linked with uterine aging during a woman's life. Nevertheless, inflammasome-containing EVs with specific Nik-related kinases present only in the reproductive system have been detected in the CSF and serum of replicative senescent rats. These inflammasomes can trigger an innate immune response in senescent female reproductive organs and then the brain (Raval et al., 2019). This result indicates that women with replicative aging are more susceptible to neurodegenerative diseases and that EVs might be the pacemakers of female aging, delivering information from the ovaries to the whole body. By monitoring the cargos and pathways of these EVs in the reproductive system with aging, we will be able to elucidate the trajectories of aging transmission in female individuals, predict pathological changes, and intervene in the occurrence of age-related diseases. These findings show that for fertility preservation, we can increase the reproductive rate and quality and decrease infertility and reproductive abnormalities through age-related EV control.

5 | EXPLOITING SENESCENCE FOR THERAPEUTICS

5.1 | Targeting senescence through senotherapy

There is a growing consensus that researching and developing therapeutic interventions for age-related disorders is necessary to alleviate the severe economic, healthcare and humanitarian challenges of the global aging society (Zhao & Stambler, 2020). Our aging population must cope with increasing threats of senescence-related dysfunction, among which mortality is the top concern and the most challenging. COVID-19 results in markedly higher in-hospital mortality in patients with advanced chronological age than in younger patients, possibly due to involvement of CD26 and ACE-2 (Koff & Williams, 2020; Li et al., 2020a; Radzikowska et al., 2020) (two host receptors that show a significant association with senescence (Li et al., 2018; Takeshita et al., 2018)). In experimental animals, inhibition of cellular senescence with the transgenic suicide gene INK-ATTAC can expand lifespan and healthspan through inducible elimination of $p16^{INK4a}$-positive senescent cells upon drug treatment (Baker et al., 2011). Using small molecules to therapeutically target exclusive markers of aging cells will likely have positive effects on the quality of life and the burden of age-related diseases. Hence, there is a growing interest in the development of antiaging treatment, termed senotherapy (Figure 4). In principle, senotherapeutic strategies can be broadly classified into two categories: senolytic strategies (antiaging strategies for selective removal of senescent cells (Jeon et al., 2017)) and senomorphic strategies (antiaging strategies that block senescent phenotypes by inhibiting the SASP without compromising cell death (Short et al., 2019)).

5.1.1 | Senolytics

Senolytic therapy specifically kills senescent cell populations depending on apoptotic resistance, an essential hallmark of cellular senescence. Using apoptosis-inducing drugs, several intracellular prosurvival pathway members have been targeted to

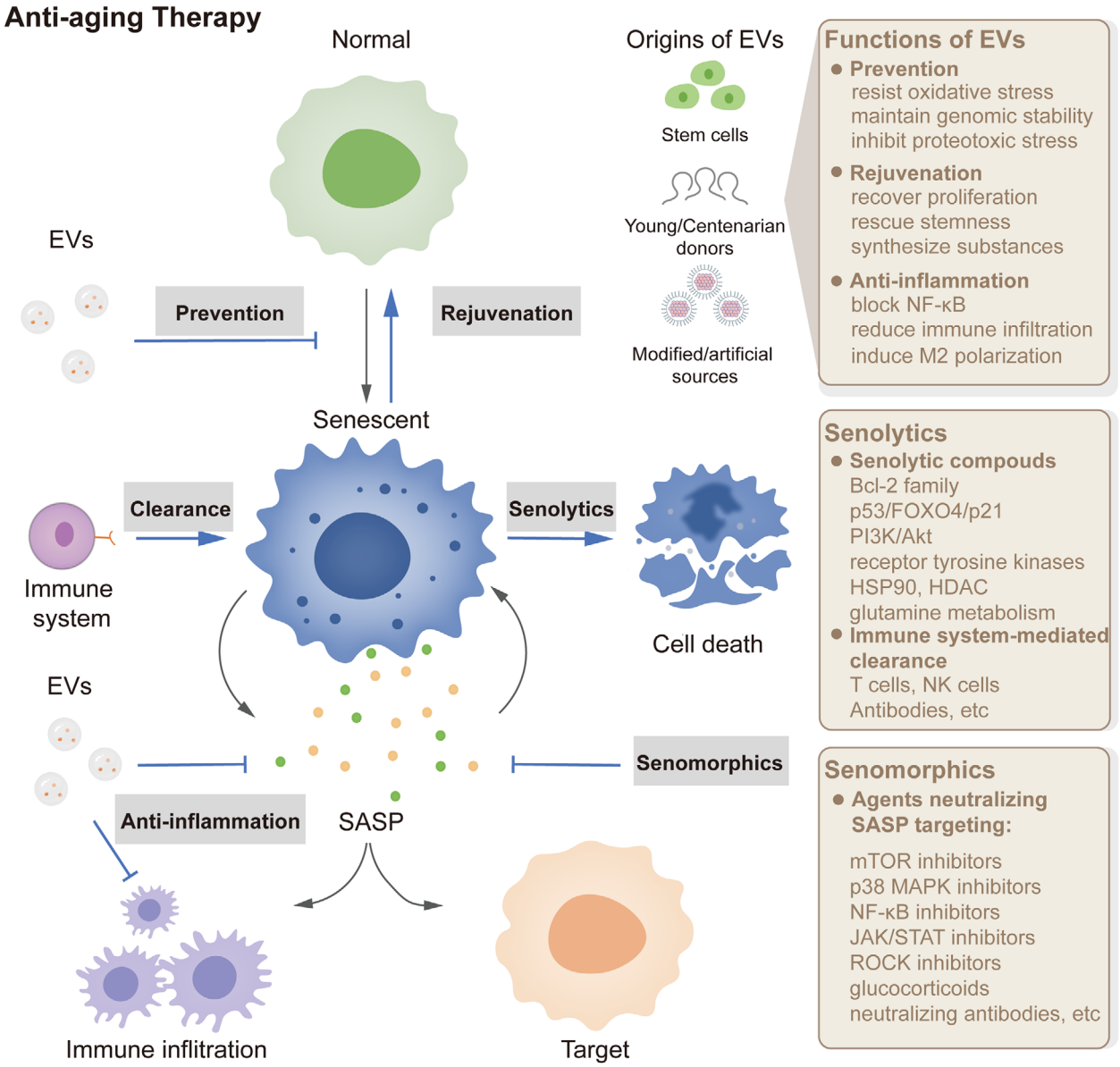

FIGURE 4 Approaches for exploiting senescence for therapeutics: senolytics, senomorphics and EV-mediated regeneration and rejuvenation. There is a growing interest in developing antiaging therapies, termed senotherapies. Senotherapeutic strategies can be traditionally classified into two categories: senolytic strategies (antiaging strategies for selective removal of senescent cells, including compound treatment and immune system-mediated clearance) and senomorphic strategies (antiaging strategies that block senescent phenotypes by inhibiting the SASP without compromising cell death). Recently, EVs, as next-generation candidates for antiaging therapy, have been well studied for their preventive, rejuvenating and anti-inflammatory effects on senescence. There are several natural sources (from stem cells, young or centenarian donors), modified sources and artificial sources of therapeutic EVs

eliminate senescent cells, including Bcl-2 family members (including Bcl-2, Bcl-W, and Bcl-X_L), p53/FOXO4/p21 pathway members, PI3K/Akt pathway members, receptor tyrosine kinases, HSP90, HDACs, and glutamine metabolism pathway members (Song et al., 2020).

The senolytic agent ABT-737 and its orally bioavailable formulation ABT-263 (navitoclax), which belong to the class of BH3 mimetics, can inhibit Bcl-2, Bcl-W, and Bcl-X_L and initiate apoptosis of senescent cells through a mitochondria-dependent pathway (Basu, 2021). The highly potent and selective Bcl-X_L inhibitors A-1155462 and A-1331852 have also been proven to have proapoptotic ability but less hematological toxicity than navitoclax (Zhu et al., 2017). In multiple senescence events, the transcription factor FOXO4 is upregulated and interacts with p53 in the nucleus, preventing apoptosis. Administration of a peptide of FOXO4, FOXO4-DRI, can perturb this interaction and cause p53 nuclear exclusion and cell-intrinsic apoptosis in vitro and in vivo (Baar et al., 2017). In senescent liver cancer cells with p53 mutations, the antidepressant sertraline exerts a selective proapoptotic function via inhibition of the DNA-replication kinase CDC7 and suppression of mTOR signaling (Wang et al., 2019). In addition, quercetin and fisetin, two natural flavonoids, have also been shown to have senolytic properties in the modulation of

several pathways, such as the PI3K/Akt/mTOR and NF-κB pathways (Ngoi et al., 2021). There exists a classic senolytic cocktail of dasatinib, a tyrosine kinase inhibitor, plus quercetin (D+Q), which has been well studied in the elimination of a wide range of senescent cells (Zhu et al., 2015). The combination of D+Q treatment can alleviate physical dysfunction and improve lifespan and healthspan in naturally aged mice by reducing the numbers of senescent cells and the levels of cytokines (Xu et al., 2018a). This senolytic combination has been verified to be effective in phase I clinical trials for diabetic kidney disease (NCT02848131) and idiopathic pulmonary disease (NCT02874989) (Hickson et al., 2019; Justice et al., 2019). In apoptosis, HSP90 is an important chaperone protein that induces the stabilization and degradation of proteins and impacts mTOR and NF-κB signaling. Its primary inhibitor 17-DMAG is a part of several classes of senolytics that delay age-related pathologies in progeroid mice (Fuhrmann-Stroissnigg et al., 2017). The HDAC inhibitor panobinostat has also been repurposed to target persistent senescent cells that accumulate after chemotherapy due to the link between senescence and histone deacetylation (Samaraweera et al., 2017). Recently, glutamine metabolism has been noted for its association with senescence. Glutaminase 1 (GLS1) not only mediates glutaminolysis but also promotes senescent cell survival, inhibition of which can clear senescent cells and improve age-related disorders in geriatric mice (Johmura et al., 2021).

In the aging microenvironment, senescent cells can be subjected to the intrinsic immunosurveillance system, which presents a suppressive trend with age. Restoring the ability of the immunosurveillance system or using modified immune cells (such as CAR-T cells (Amor et al., 2020)) might be successful in the targeted clearance of senescent cells from tissues (Di Micco et al., 2021). For example, to enhance the cytotoxic activity of NK cells, polyinosinic-polycytidylic acid (polyI:C, a TLR3 ligand) has been administered to induce IFN-γ and activate the NK cell receptor NKG2D, resulting in clearance of senescent activated stellate cells and amelioration of liver fibrosis (Krizhanovsky et al., 2008).

5.1.2 | Senomorphics

Senomorphic therapy interferes with the proinflammatory nature of senescent cells and normalizes the SASP process in the aging microenvironment through the regulation of various biochemical pathways involving mTOR, p38 MAPK, NF-κB, JAK/STAT, ROCK, glucocorticoid receptors, and neutralizing antibodies (Prasnikar et al., 2021).

As stated previously, several stress response signaling cascades are activated to induce the secretion of inflammatory components, including NF-κB, C/EBPβ and their upstream components. Through efficient blockade of the signaling cascades or direct inhibition of the activity of SASP components, the amplifying outcomes of senescence can be ameliorated in the aging microenvironment. mTOR, one of the most critical upstream signaling pathways in the process of cellular senescence and the SASP, can be inhibited with rapamycin and its analogs, such as RAD001, and can present a senomorphic function. In normal human fibroblasts and breast cells, rapamycin can suppress IL-6 secretion, selectively decrease IL-1α translation and diminish NF-κB transcriptional activity (Laberge et al., 2015). In Nrf2KO mice and WI-38 fibroblasts, it can also significantly decrease IL-6 and IL-1 transcription through Stat3 inhibition in a Nrf2-independent mechanism (Wang et al., 2017a). Inhibition of another essential SASP-modulated pathway, the p38 MAPK pathway, with SB203580 and the next-generation compounds UR-13756 and BIRB796 has also been proven to reduce NF-κB transcriptional activation, thereby inhibiting the paracrine effects of senescence (Alimbetov et al., 2016; Freund et al., 2011). In addition to NF-κB, the JAK/STAT pathway can sustain cytokine production through C/EBPβ activation. In human preadipocytes, endothelial cells and aged mice, the JAK1/2 inhibitor ruxolitinib attenuates SASP-related secretion and reduces systemic inflammation, leading to improvements in metabolic function and physical capacity (Xu et al., 2015). However, given the diversity of the signaling pathways of the SASP in different cell types, interference with any one pathway may not affect all components.

Another strategy of senomorphics is to achieve the antiaging goal through direct modulation of NF-κB binding and activation. Among senomorphics with this strategy, metformin has been used the most extensively. Metformin, an FDA-approved oral hypoglycaemic medication for T2DM, was the first drug to be tested in a large clinical trial for age-related diseases termed the TAME (Targeting Aging by Metformin) trial (Barzilai et al., 2016). It can significantly interfere with IKK/NF-κB activity and reduce NF-κB nuclear translocation and activation to decrease SASP-related production independently of AMPK (Moiseeva et al., 2013). Additionally, it inhibits mTOR activity and modulates certain downstream cellular effects to extend lifespan across multiple model systems (Soukas et al., 2019). However, its mechanisms in aging and the SASP are not yet clearly defined. The SASP can also be regulated by a class of anti-inflammatory steroid hormones, the glucocorticoids. Both corticosterone and cortisol are efficacious in suppressing the SASP via glucocorticoid receptors by preventing IL-1α positive feedback and inhibiting NF-κB binding and transactivation (Laberge et al., 2012). Although reduction of SASP component production is an aspect of traditional senomorphic treatment, the release process of SASP is rarely targeted for interference. Recently, it was reported that inhibition of Rho/Rho kinase with CT04 and Y27632 may not only alter the morphology of senescent cells but also affect the secretion of senescent cells via changes in the ER/Golgi system (Simay Demir et al., 2021). Alternatively, more direct inhibition can be achieved by specific neutralizing monoclonal antibodies against SASP factors, such as MABp1 for IL-1α, ABX-IL-8 for IL-8 and olokizumab for IL-6 (von Kobbe, 2019).

Collectively, these strategies take advantage of a broad spectrum of cellular pathways, indicating that senescence can be modulated and controlled via numerous avenues. However, some of these drugs are in fact repurposed anticancer medicaments, the adverse effects of which cannot be ignored. Additionally, inhibition of senescence through clearance or disruption does not directly reverse aging. The efficacy of these senotherapies is still limited for rejuvenation and regeneration. Hence, other antiaging therapies urgently need to be developed creatively from novel perspectives of aging and translated rapidly from the bench to the bedside.

5.2 | Achieving rejuvenation and regeneration via EVs

Extracellular vesicles are able to program their target cells to follow a Trojan-horse approach, which enables them to exert pathogenic and curative effects depending on their source cells. In the aging microenvironment, EVs can transmit aging phenotypes from senescent cells in amplifying ways. However, they can also be cell-free therapeutic materials and tools with no risk of tumour induction, and they show a low probability of immune rejection during regeneration and rejuvenation (Adamiak et al., 2018; Zhai et al., 2020). It has been determined that EVs are next-generation candidates for use in achieving antiaging goals.

5.2.1 | Natural sources of EVs

There are natural, modified and artificial sources of therapeutic EVs (Table 3). Among natural sources, stem cells are the primary sources, and young donors are the main population group supplying these sources. EVs originating from stem cells contain diverse cargos that can execute antioxidative stress and anti-inflammatory activities in order to induce antiaging effects (Figure 4).

Stem cell-derived therapeutics

UV radiation is an extrinsic factor of skin photoaging that disturbs human dermal fibroblasts (HDFs) via ROS production and extracellular matrix degradation (Xu et al., 2020). Pretreatment and posttreatment with EVs from human umbilical cord mesenchymal stem cells (MSCs), adipose-derived stem cells (ADSCs), and induced pluripotent stem cells (iPSCs) are linked to a broad range of antiphotoaging functions, including fibroblast proliferation, collagen biosynthesis, DNA repair and UV protection (Choi et al., 2019; Deng et al., 2020; Oh et al., 2018b; Xu et al., 2020). Because of their antioxidant proteins, such as glutathione peroxidase 1, superoxide dismutase and catalase, EVs from the aforementioned cell sources are well suited to control the mitochondrial membrane and clear accumulated ROS (Deng et al., 2020; Xu et al., 2020). Human iPSCs show stronger antiphotoaging abilities than mesenchymal stem cells, with 16-fold more EV production (Liu et al., 2019a).

However, in wound healing and inflammaging diseases, inhibition of a robust immune response is the critical mechanism of stem cell-based EV treatment (Bian et al., 2020). After myocardial infarction or oxidant treatment, senescent cardiomyocytes are enriched during local inflammatory responses (Deng et al., 2019; Zhu et al., 2019). The exosomal lncRNA MALAT1 derived from MSCs inhibits the NF-κB/TNFα pathway to rescue myocytes from aging and increases the left ventricular ejection fraction and fractional shortening (Zhu et al., 2019). ADSC exosome treatment activates S1P/SK1/S1PR1 signaling and promotes macrophage M2 polarization to mitigate cardiac damage (Deng et al., 2019). ADSC exosomes can also downregulate inflammatory cytokines and PGE2 expression in OA osteoblasts (Tofino-Vian et al., 2017). Systemic inflammaging is associated with the loss of hypothalamic stem cells with increased age. Core treatment using exosomal miRNAs from hypothalamic stem cells can inhibit NF-κB function and restore GnRH secretion, consequently reducing physiological deficits and extending lifespan (Meyer & Yankner, 2017; Zhang et al., 2017).

EVs from stem cells also inherit the ability to rescue the stemness of target stem cells. Human embryonic stem cell-derived EVs can alleviate the senescence of tissue-resident stem cells, such as BMSCs, to prevent bone loss with aging (Gong et al., 2020). MSCs can also rescue themselves from senescence and improve stemness through miR-302b and HIF-1 activation (Mas-Bargues et al., 2020). To treat neurodegenerative diseases, EVs obtained from stem cells mainly transfer miRNAs to promote lysosome resumption and cytotoxic protein degradation (Elia et al., 2019; Hu et al., 2020). Interestingly, EVs noninvasively harvested from urine-derived stem cells contain collagen triple-helix repeat-containing 1 (CTHRC1) and osteoprotegerin (OPG), which effectively reduce age-related bone loss (Chen et al., 2019b). Treatment with these EVs is another regenerative therapy for osteoporosis patients who have unlimited access to the key treatment component.

Young donor-derived therapeutics

In bone marrow transplantation, donor age is an essential concern because of engraftment failure associated with hematopoietic stem cell (HSC) senescence. Nonetheless, young donor-derived vesicles can rejuvenate aged HSCs to improve autologous transplantation (Kulkarni et al., 2018). EVs from young donor serum contain high levels of nicotinamide phosphoribosyltransferase (eNAMPT) and glutathione-related protein (GSTM2), both of which play roles in antioxidation, and their levels decline with age. NAMPT enhances NAD^+ biogenesis and has antiaging effects on the hypothalamus, pancreas, retinas, and hippocampus.

TABLE 3 Novel antiaging treatments via extracellular vesicles

Treatments and sources	Cargo(s)	Subjects	Mechanism(s) and Effect(s)	Ref.
Stem cell-derived therapeutics				
Hypothalamic stem cells	miRNAs	Aged mice	Blocked NF-κB activation and restored GnRH secretion in neurons; Reduced age-related physiological deficits and extended lifespan	(Zhang et al., 2017)
Human umbilical cord MSCs	LncRNA MALAT1	Cardiomyocytes with H_2O_2-induced aging; Mice with D-galactose-induced aging	Inhibited the NF-κB/TNFα pathway; increased TERT expression; Increased the left ventricular ejection fraction and fractional shorting in aging mice	(Zhu et al., 2019)
Human umbilical cord MSCs	/	UVB-irradiated HDFs	Reduced production of ROS and increased glutathione peroxidase; Inhibited UVB-induced senescence and induced fibroblast proliferation	(Deng et al., 2020)
ADSCs	/	IL-1β-treated OA osteoblasts	Reduced inflammatory mediators including IL-6 and prostaglandin E2; Controlled mitochondrial membrane stability and reduced oxidative stress	(Tofino-Vian et al., 2017)
ADSCs	miRNAs Proteins	UVB-irradiated HDFs	Induced senescent fibroblast proliferation and DNA repair and attenuated aging; Suppressed the expression levels of MMP-1, 2, 3, and 9 and enhanced collagen; Provided protection from UVB irradiation and enhanced recovery from skin photoaging	(Choi et al., 2019)
ADSCs	/	Myocardial infarction mice, myocytes, fibroblasts, and macrophages	Activated S1P/SK1/S1PR1 signaling and promoted M2 polarization; Suppressed cardiac dysfunction, apoptosis, fibrosis, and the inflammatory state	(Deng et al., 2019)
ADSCs	/	Photoaging mice and fibroblasts	Attenuated macrophage infiltration and ROS production; Promoted cell proliferation and decreased skin wrinkles	(Xu et al., 2020)
Human iPSCs	/	Aged and UVB-irradiated HDFs	Reduced the expression levels of SA-β-Gal and MMP1, 3; Restored collagen type I and ameliorated UVB-induced skin aging	(Oh et al., 2018b)
Human urine-derived stem cells	CTHRC1; OPG	Osteoporotic mice	Enhanced osteoblastic bone formation and suppressed bone resorption	(Chen et al., 2019b)
Human embryonic stem cells	miR-200a	Aged mice with pressure-induced ulcers; Senescent endothelial cells	Downregulated Keap1 and activated the Nrf2 pathway; Enhanced EC proliferation and angiogenesis; Accelerated wound closure and ameliorated senescent phenotypes	(Chen et al., 2019a)
Human embryonic stem cells	4122 proteins	Senescent BMSCs	Rescued the function of senescent BMSCs and promoted proliferation; Induced osteogenic differentiation and reduced age-related bone loss	(Gong et al., 2020)
Embryonic stem cells	Several miRNAs	Vascular dementia mice	Alleviated senescence and loss of hippocampal stem cells; Reduced cognitive decline by activating mTORC1 and promoting TFEB translocation and lysosome resumption	(Hu et al., 2020)
Embryonic stem cells	SMAD4, 5	Aged mice	Activated MYT1 and improved HIF-2α, NAMPT and SIRT1 levels; Alleviated hippocampal stem cell senescence, reversed cognitive impairment	(Hu et al., 2021)
BMSCs	Neprilysin	APP/PS1 AD mice	Reduced Aβ plaques and dystrophic neurites in the cortex and hippocampus	(Elia et al., 2019)

(Continues)

TABLE 3 (Continued)

Treatments and sources	Cargo(s)	Subjects	Mechanism(s) and Effect(s)	Ref.
Human decidua-derived MSCs	/	High glucose-treated fibroblasts; Diabetic mice	Rescued the senescent state via inhibition of RAGE and activation of Smad; Accelerated collagen deposition and enhanced diabetic wound healing	(Bian et al., 2020)
Amniotic fluid MSCs	miR-21	Mice with premature ovarian dysfunction	Increased total follicular counts and AMH levels and restored regular oestrous cycles by modulating the PTEN and caspase 3 apoptotic pathway	(Thabet et al., 2020)
Amniotic fluid MSCs	miR-320a	Mice with premature ovarian insufficiency; POI human granulosa cells	Decreased SIRT4 and ROS levels; Improved proliferation, inhibited apoptosis and elevated ovarian function	(Ding et al., 2020)
Dental pulp stem cells	miR-302b	Senescent dental pulp stem cells	Improved stemness through HIF-1α and OSKM upregulation; Switched energetic metabolism toward glycolysis through the ERK pathway	(Mas-Bargues et al., 2020)
Modified stem cell therapeutics				
GAG-coated matrix vesicles	/	BMSCs	Promoted differentiation of osteoblasts from hBMSCs and reduced osteoclasts	(Schmidt et al., 2016)
Serially extruded human iPSCs	Oct4 Nanog	Naturally senescent HDFs	Reduced the senescent state and ameliorated skin aging	(Lee et al., 2020)
SHS-coated MS Exos	/	Photoaging mice	Enhanced skin absorption of exosomes by creating microchannels; Reduced wrinkles and promoted extracellular matrix constituents	(Zhang et al., 2020)
BMSC Exos with aptamer	miR-26a	Mice with OVX-induced osteoporosis; Femur fracture mouse model	Promoted bone regeneration and accelerated bone healing; Enhanced bone targeting and bone generation	(Luo et al., 2019)
MSC Exos in silk fibroin hydrogel	miR-675	Aged animal model; H_2O_2-treated H9C2 cells	Prolonged the half-life of exosomes in vivo; Reduced aging hallmarks and promoted blood perfusion	(Han et al., 2019b)
ACE2-primed endothelial progenitor cell Exos	ACE2 miR-18a	Aging ECs	Protected ECs from hypoxia/reoxygenation-induced injury through downregulation of Nox2/ROS	(Zhang et al., 2018a)
LPS-preconditioned BMSC Exos	/	MI mouse model	Increased M2 macrophage polarization upon LPS stimulation; Attenuated inflammation and cardiomyocyte apoptosis	(Xu et al., 2019)
Cytokine-preconditioned MSC Exos	/	APP/PS1 AD mice	Reduced activation of microglia and increased the dendritic spine density; Induced immunomodulatory and neuroprotective effects	(Losurdo et al., 2020)
Predifferentiated MSC Exos in 3D-printed titanium scaffolds	/	BMSCs	Induced osteogenesis and regenerated bone tissue through the PI3K/Akt and MAPK pathways	(Zhai et al., 2020)
Other cell-derived therapeutics				
Wnt4-transgenic TECs	Wnt4; miR-27b	TECs with steroid-induced aging	Counteracted thymic adipose involution; Reduced age-related thymic dysfunction	(Banfai et al., 2019)
Endothelial cells	/	Senescent fibroblasts; Diabetic mice	Reduced senescent phenotypes through YAP translocation and the PI3K/Akt pathway and promoted wound healing in diabetic mice	(Wei et al., 2020b)
3D human dermal fibroblasts	TIMP-1	Photoaging mice	Induced collagen synthesis and antiaging effects	(Hu et al., 2019)

(Continues)

TABLE 3 (Continued)

Treatments and sources	Cargo(s)	Subjects	Mechanism(s) and Effect(s)	Ref.
Young donor-derived therapeutics				
Young mouse serum	eNAMPT	Aged mice	Enhanced NAD+ biosynthesis and controlled oxidative stress; Enhanced physical activity and extended lifespan	(Yoshida et al., 2019)
Primary fibroblasts of young human donors	GSTM2	HGPS fibroblasts; Aged mice	Reduced senescent phenotypes by restoring antioxidant capacity; Prevented lipid peroxidation	(Fafia, Rodriguez-Navarron-Labora et al., 2020)
Young human serum	/	DOX-treated cardiomyocytes	Retarded cellular senescence through the miR-34a/PNUTS pathway	(Liu et al., 2019b)
Young mouse serum	miRNAs	Aged mice	Rejuvenated thymic aging and enhanced thymic negative selection	(Wang et al., 2018)
Young mouse serum	miRNAs	Aged mice	Reduced aging gene expression and induced telomerase gene expression	(Lee et al., 2018)
Centenarian donor-derived therapeutics				
Centenarian fibroblasts	RNAseH2C enzyme	Young HDFs	Reduced inflammatory cytokines and upregulated anti-inflammatory enzymes; Induced M2 polarization and genomic stability	(Storci et al., 2019)
Modified nanomedicines				
Catalase-loaded exosomes	Catalase	PD mice	Prolonged circulation time and induced neuroprotective and antioxidant effects	(Haney et al., 2015)

GSTM2 enhances intrinsic glutathione-S-transferase activity to scavenge ROS and prevent lipid peroxidation (Fafian-Labora et al., 2020b; Yoshida et al., 2019). With regard to anti-inflammation, EVs from young mouse serum can rejuvenate the aging thymus and enhance central negative selection signals to reinforce central tolerance. Then, they can ameliorate peripheral and CNS self-immune responses by passing through the blood-brain barrier (Wang et al., 2018). miRNAs from young donor-derived EVs can also reduce telomere shortening and DNA damage (Lee et al., 2018; Liu et al., 2019b).

Centenarian donor-derived therapeutics

The aging microenvironment, which is faced with external and internal risk factors, is equipped with considerable antioxidant machinery to eliminate ROS in endothelial EVs and HUVEC EVs, which mainly contain antioxidant enzymes and peptides as well as NADPH-synthesizing enzymes (Bodega et al., 2017, 2018). Given this adaptive ability, centenarian donor-derived EVs may have more potent antioxidant agents to maintain organ and systemic balance. In fibroblasts of centenarians, EVs contain high levels of the enzyme RNAseH2C, which can restrain DNA damage-induced inflammation and ameliorate age-associated low-grade chronic inflammation (Storci et al., 2019). This phenomenon also implies that the antioxidant machinery is another controllable node of pathological transformation in the aging microenvironment. To some degree, intervention with antioxidant elements can support microenvironment stability.

5.2.2 | Modified/artificial sources of EVs

Although EVs purified from cells or the serum of healthy donors can directly exert profound effects for rejuvenation, there are still inefficiency issues with natural cell-free therapy, including low production, weak targeting, short half-lives, and fast clearance. To address these problems, modified and artificial EVs should be designed well to promote the sensitivity, specificity, and safety of antiaging therapy.

Increasing the yield of EVs from stem cells is the first step toward promoting antiaging sensitivity. A serial extrusion method in which human iPSCs are passed through membrane filters with diminishing pore sizes can generate more cell-engineered nanovesicles, showing an excellent ability to reduce senescence hallmarks of naturally aging HDFs and ameliorate skin aging (Lee et al., 2020). In addition to quantity-promoting methods, methods that produce high-quality EVs are crucial and valuable for clinical applications. Coatings on implants can facilitate EV penetration through tight physiological barriers to enable the EVs to subsequently exhibit dynamic functions. A skin coating made from SHSs (from the spicules of the marine sponge *Haliclona*) and used with MSC-derived exosomes enhances skin penetration by creating microchannels, exhibiting significant antiphotoaging

effects with slight skin irritation (Zhang et al., 2020). Another coating is made with sulfated glycosaminoglycans (GAGs), which synergize with EVs to strengthen extracellular matrix formation and mineralization in age-related bone loss (Schmidt et al., 2016). Preconditioned cells and EVs also have roles in quality improvement. MSCs osteogenically predifferentiated for 10–15 days can be efficiently harvested as sources of osteogenic exosomes, which induce bone tissue regeneration through osteogenic miRNAs after loading onto 3D-printed titanium alloy scaffolds (Zhai et al., 2020). Cells cultured in vitro as 3D spheroids can also produce high-quality EVs (Hu et al., 2019). Directly encapsulating antiaging medicines into EVs as artificial nanomedicines is a method for developing high-affinity and high-quality antiaging systems. For example, a new exosomal delivery system for catalase, an antioxidant, has visibly neuroprotective effects in PD after intranasal delivery (Haney et al., 2015).

Prolonging the half-life of functional EVs would address sensitivity and specificity problems by preventing ectopic distribution and clearance. Age- and tissue-specific miRNAs, such as miR-675, mainly remain and function in muscle tissue; these miRNAs can be encapsulated in EVs and used to decorate silk fibroin hydrogel in order to increase the retention time in muscle cells and thereby cure ischemic diseases (Han et al., 2019b). Using these methods, the targeting problem can also be resolved. Through combination with specific aptamers, EVs containing miR-26a from BMSCs can be directionally driven to bone marrow to accelerate bone healing after fracture (Luo et al., 2019).

For safety, side effects, primarily those involving excessive inflammation, should be minimized. After myocardial infarction, cardiomyocytes treated with EVs from lipopolysaccharide (LPS)-preconditioned BMSCs can reduce apoptosis and inflammation by mediating macrophage M2 polarization (Xu et al., 2019). In AD, cytokine-preconditioned MSCs produce immunomodulatory and neuroprotective EVs, achieving antiaging and anti-inflammatory goals after intranasal delivery (Losurdo et al., 2020).

In conclusion, as burgeoning biomaterials for antiaging therapy, EVs play roles in treating age-related diseases. However, questions related to their tumorigenicity and manoeuvrability should be addressed. With the diversity of EVs and their possible applications in nanomedicines, it is anticipated that more specific senolytic and senomorphic strategies can be developed that rely on EVs.

6 | CONCLUSION AND PERSPECTIVES

Cellular senescence can be a potential launching point for various diseases, not only because of its association with organ/systemic aging but also because of its nature. As a relatively stable intermediate state between survival and death, senescence is associated with specific markers and cell morphological changes, which is convenient for qualitative and quantitative detection and evaluation (Hernandez-Segura et al., 2018). There are significant differences and hidden weaknesses in chromatin structures, gene expression, and metabolic profiles of senescent cells compared with nonsenescent cells, which can be monitored by the immune system (Liu et al., 2020; Wang et al., 2019). Moreover, for tumours, the characteristics of cell cycle arrest can be leveraged for a physiological anticancer effect.

In this review, we have discussed the characteristics of the aging microenvironment for the first time, highlighting new ideas that might be developed for the treatment of age-related diseases. On the one hand, in view of the instability of the aging microenvironment, prompt prevention and exclusion of interfering factors must be the focal points and be managed accordingly. On the other hand, considering the amplification effect of the aging microenvironment, inhibiting the secretion of harmful molecules and cutting off routes of aging transmission can buffer the adverse effects of the inflammatory microenvironment. In addition, in light of the transformative nature of the aging microenvironment, exploring the key mechanism of transformation and intervening in key events can effectively forestall and reverse the establishment and progression of pathological states.

Accordingly, EVs play undeniable roles in cellular senescence and age-related diseases and can be used in promising approaches to identify and therapeutically target senescent cells. However, there are still several challenges associated with the development of this young but emerging field of research, including a lack of optimal and verified experimental models, details on the biogenetic mechanism, a comprehensive age-related EV atlas and a rapid capture and visualization system. Given the long aging period of humans, fast-aging model organisms, such as *Caenorhabditis elegans* and yeasts, and animals, such as naturally aging mice or gene-edited mice, are widely used in geroscience. However, they do not faithfully recapitulate human states due to the complex multicell and multiorgan networks of senescence. Better-suited animal models (e.g., nonhuman primates) or human organoids are required for research on the topics of EVs and aging. As secretion marks the onset of EV function, clarifying the detailed molecular mechanism of EV secretion may be helpful for elucidating the aging transmission pathway and developing efficient therapeutic interventions. In addition, given the heterogeneity of senescence and EVs, creation of a comprehensive atlas of age-related EVs from different cells under different stresses would be enormously helpful for this field. It is possible that a specific ageotype may indicate an individual physiological and pathological status as a more distinctive biomarker than others now available. Senescence-associated EVs can now be detected in blood, urine, CSF, and saliva, which provides us with new inspiration for diagnostic and prognostic tools using liquid biopsy (Machida et al., 2015; Perrotte et al., 2020; Santelli et al., 2019). Due to the prion-like behaviour of EVs, in vivo imaging techniques have been designed, such as techniques involving EV labelling combined with CT that longitudinally and quantitatively trace aberrant neurons in the brains of PD, AD, and stroke patients

(Perets et al., 2019). However, a full understanding of which cells are senescent and which cells secrete and take up aging EVs in vivo is lacking owing to the unsuitable and inaccurate capture and visualization systems.

To develop clinical antiaging therapies, safety should first be considered, followed by sensitivity and specificity. Because of the specificity of immune recognition, senolytic CAR-T and senescent CD153 vaccines have been proven feasible and efficient (Amor et al., 2020; Yoshida et al., 2020). This finding suggests that immune-modified EVs may be able to preferentially target senescent cells and tissues to promote senotherapy. However, before EV transplantation is performed, the routes of administration, reasonable dosages, dosing intervals, and host nutritional status should be strictly verified. EVs can be considered promising materials and tools for antiaging treatments after rigorous trials.

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