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Review article

Role of mesenchymal stromal cell secretome on recovery from cellular senescence: an overview

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ABSTRACT

Cellular senescence is intricately linked with numerous changes observed in the aging process, including the depletion of the stem cell pool and the decline in tissue and organ functions. Over the past three decades, efforts to halt and reverse aging have intensified, bringing rejuvenation closer to reality. Current strategies involve treatments using stem cells or their derivatives, such as the secretome. This article aims to highlight key points and evaluate the utilization of secretome derived from mesenchymal stromal cells (MSCs) as an antisenescent approach. Employing a quasi-systematic research approach, the authors conducted a comprehensive analysis based on a search algorithm targeting the *in vitro* effects of MSC-derived secretome on rescuing cells from a senescent state. Reviewing 39 articles out of 687 hits retrieved from PubMed and Scopus without a time limit, the authors synthesized information and identified common types of MSC-tissue sources utilized (including bone marrow-MSCs, umbilical cord-MSCs, iPSC-derived MSCs, adipose tissue-MSCs, dental pulp-MSCs, amniotic membrane-MSCs, placenta-MSCs, gingival-MSCs, urine-MSCs, and commercially available MSC lineages) from both human and other species (such as mice and rats). The authors also examined the forms of secretome tested (including conditioned media and extracellular vesicles), the cell types treated (MSCs or other cell types), methods/biomarkers of monitoring senescence/rejuvenation, and the mechanisms involved. Ultimately, this review underscores the proof-of-principle of the beneficial effects of MSC-derived secretome in reversing cellular senescence across various cell types. Such insights might aid the scientific community in designing improved *in vitro* and *in vivo* assays for future research and clinical validation of this promising cell-free therapy.

Introduction

Aging is characterized by a gradual decline in both mental and physical functional abilities and is considered the primary risk factor for most diseases [1,2]. In mammals, this process occurs heterogeneously across organ systems, leading to progressive deterioration and eventual tissue dysfunction [3]. The hallmarks of aging encompass genome instability and telomere attrition, epigenetic alterations, dysregulation of proteostasis and nutrient sensing, impaired macroautophagy, mitochondrial dysfunction, depletion of the stem-cell pool, compromised cell-cell communication, dysbiosis, chronic inflammation, and cellular senescence [4]. The latter is a multifaceted process involving various alterations, including morphological changes, metabolic adaptations, cell cycle arrest, chromatin reorganization, and the development of a senescence-associated secretory phenotype (SASP) [5].

Hayflick and Moorhead (1961) [6] were the first to describe cellular senescence as an irreversible, nondividing but metabolically active state. Presently, it is recognized as a mechanism of cell cycle arrest following harmful or stressful stimuli. Senescence can be categorized based on the triggering stimulus as premature (induced by DNA damage), replicative, stress-induced, or oncogene-induced senescence [7,8]. Senescent cells remain metabolically active even after exhausting their replicative potential *in vitro*. They fail to respond to mitogenic signals and produce a complex secretome that significantly impacts the tissue microenvironment [5,9]. While cellular senescence plays a role in physiological events such as tumor suppression, tissue repair, and embryogenesis [10], excessive, chronic, and aberrant accumulation of senescent cells impairs tissue and organ repair and regenerative capacity, fostering a proinflammatory microenvironment that contributes to secondary senescence associated with aging

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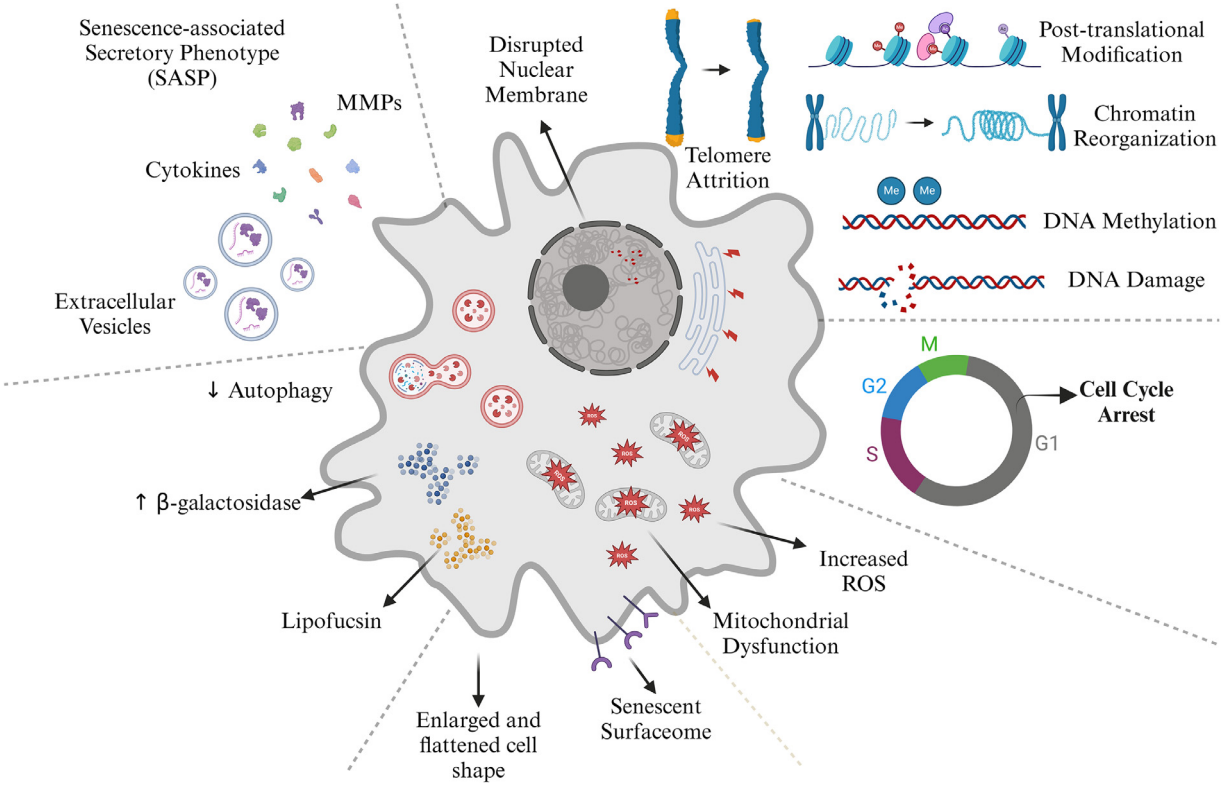

Fig. 1. Cellular alterations associated with senescence that are used as biomarkers. Senescent cells present morphological changes with enlarged and flattened morphology. Genomic instability is observed involving DNA damage, telomere attrition, and dysfunction of the nuclear membrane. Epigenetic changes include chromatin remodeling, DNA methylation, and histones post-translational modifications. Metabolic dysfunction was detected with alterations in the lysosomal compartment characterized by an increase in β-galactosidase, lipofuscin granules, and reduction of autophagy, in addition to mitochondrial damage and ROS increase. Cell cycle arrest and alteration of the secretory pattern of senescent cells, called SASP, were also observed. Created with BioRender.com. (Color version of figure is available online.)

and aging-related diseases such as osteoarthritis, Alzheimer's disease, frailty, idiopathic pulmonary fibrosis, and cancer [7,11,12].

Cell therapy is emerging as a rejuvenation strategy aimed at reversing cellular aging and treating age-related diseases. Mesenchymal stromal cells (MSCs) represent promising candidates for this purpose, as they can be isolated from various tissues, possess multipotent characteristics, exhibit self-renewal capacity, and demonstrate immunomodulatory and angiogenic properties. Furthermore, MSCs have low immunogenicity when transplanted allogeneically [13,14]. MSC-based therapy holds the potential to rejuvenate skin, the immune system, and cartilage [15–18].

In this context, the MSC secretome—comprising extracellular biomolecules and extracellular vesicles (EVs) produced and released by cells has been recognized as the primary mechanism underlying their therapeutic effects. It can be obtained by selecting the supernatant of MSCs, known as conditioned medium (CM), during culture. The use of CM or EVs has emerged as an acellular or cell-free therapy alternative to MSC transplantation, aiming to circumvent limitations such as tumor formation, differentiation into undesired cell types, low engraftment rates, and immune reactions [19,20]. Both *in vivo* and *in vitro* studies have demonstrated the efficacy of CM/EVs derived from MSCs in regulating cell proliferation, viability, and migration; reducing oxidative stress and cell death; and promoting tissue regeneration [21–24].

Considering the beneficial effects of MSC treatment on senescence and aging-related diseases, as well as the therapeutic potential of MSC-derived secretome as an alternative to direct MSC use, their secretome emerges as a promising antiaging strategy. Therefore, this review article delves into key aspects related to the development of rejuvenating therapies using MSC secretome in *in vitro* assays. First, the authors provided a summary of the most commonly cited senescence biomarkers and emphasized strategies and techniques for assessing senescence. Then, employing a quasi-systematic research approach, they examined the rejuvenating effects of MSC-derived secretome on senescent cells, including MSCs and differentiated cells, and elucidated the molecular mechanisms involved in this process. Furthermore, future perspectives on research and the development of successful therapies to rescue tissues from senescence were presented.

Biomarkers of senescent cells

This section focuses on the main characteristics of the senescent phenotype utilized as biomarkers in *in vitro* studies (Figure 1). Senescent cells exhibit enlarged and flattened morphology accompanied by dysfunctional metabolism, commonly assessed through the expression and activity of β-galactosidase, a eukaryotic hydrolase found in the lysosomal compartment [25,26]. Therefore, measuring β-galactosidase activity is a straightforward technique to evaluate the increased number and size of lysosomes in senescent cells, contrasting with quiescent or differentiated cells in which they are undetectable.

The impairment of autophagy and proteostasis has also been associated with aging, leading to a gradual decline in lysosomal hydrolase activity and resulting in cellular damage [7]. In this context, lipofuscin, known as the age pigment, is widely utilized as a senescent biomarker. It comprises highly oxidized cross-linked macromolecules (including proteins, lipids, and sugars) that are neither degraded nor cleared by exocytosis, accumulating within the lysosomes and cytoplasm of long-lived postmitotic and senescent animal cells. Proliferative cells efficiently dilute lipofuscin aggregates during cell division, unlike senescent cells, which have compromised proliferation and accumulate the pigment [25,27].

Additionally, mitochondrial dysfunction stands out as a primary factor in senescence, leading to increased levels of reactive oxygen

species (ROS), which, in turn, promote cellular and DNA damage. Elevated ROS levels resulting from mitochondrial dysfunction also contribute to metabolic dysfunction and senescence. ROS induce oxidative stress, leading to telomere shortening [28], DNA damage [29], epigenetic alterations [30], and activation of tumor suppression pathways [31]. Moreover, ROS excess damages mitochondrial DNA and impairs the regular expression of mitochondrial genes involved in energy metabolism. This disruption reinforces ROS production, reduces ATP synthesis, and decreases NAD+ levels, thereby impacting energy production and DNA repair [7].

The arrest in the cell cycle is one of the most defining hallmarks of cellular senescence, characterized by an increased expression of regulatory proteins, such as p16, p21, and p53. Analyzing the number of cells in each cell cycle phase can be utilized to assess growth arrest, as senescent cells typically remain in the G1 and possibly G2 phase, unlike quiescent cells, which remain in the G0 phase [32]. Consequently, an increase in the number of cells entering the S phase could indicate a proliferative state and re-entry into the cell cycle. Thus, considering that cellular senescence entails irreversible and nondividing states, evaluating cell proliferation using techniques such as BrdU incorporation and population doubling level analysis is a fundamental criterion for assessing senescence. Furthermore, as senescent cells lose their ability to generate colony-forming units (CFU), the CFU assay serves as one of the most convenient indicators of MSC senescence [8].

Besides, DNA damage, manifested as breaks and telomere attrition, triggers senescence upon reaching a critical length. The DNA damage response (DDR) signals enable cells to recognize damaged DNA, such as double-strand breaks (DSBs), thereby halting cell cycle progression and inducing senescence [33]. In this context, γ-H2AX, formed by the phosphorylation of the Ser-139 residue of the histone variant H2AX, serves as an early cellular response to DSBs, thus acting as a sensitive marker of DNA damage and repair [34]. Additionally, 8-hydroxy-2-deoxyguanosine (8-OHdG), an oxidized nucleoside of DNA and a predominant form of free radical-induced oxidative lesions has been widely utilized as a biomarker for oxidative DNA damage [35].

Senescent cells also exhibit transcriptional alterations mediated by noncoding RNAs (ncRNAs) and epigenetic changes involving chromatin remodeling, post-translational histone modifications, and shifts in DNA methylation patterns. Regions of constitutively silenced retrotransposable elements within heterochromatin become reactivated during aging, leading to DNA instability and damage [36]. Such changes can be identified through the analysis of senescence-associated heterochromatin foci (SAHF), which are specialized domains of facultative heterochromatin contributing to the suppression of proliferation-promoting genes. Significantly, SAHFs are not associated with cells undergoing quiescence and thus do not indicate reversible cell cycle arrest [37]. However, one of the most notable features is SASP, wherein cells produce elevated levels of proinflammatory cytokines, chemokines, and metalloproteases, thereby promoting secondary senescence and accelerating aging progression [28,33,36].

Hence, studies have concentrated on modulating senescence biomarkers as an antiaging strategy. In the subsequent sections, focusing on information gleaned from articles selected through a quasi-systematic research approach, the authors will lay the groundwork for a better understanding of the rejuvenating potential of MSC secretome in various cell types, including MSCs themselves and the molecular mechanisms involved in this process.

Literature Search

The authors utilized the PubMed and Scopus search platforms and employed a Boolean search query, outlined in the subsequent sections. They selected original articles assessing the impact of MSC-derived secretome on rejuvenating senescent cells *in vitro*, elucidating the pathways implicated in this rejuvenating process, and exploring techniques/approaches for evaluating senescence status. Some *in vivo* data were mentioned to complement the *in vitro* results. The search encompassed articles up to November 30, 2023, across the disciplines of medicine, biochemistry, genetics, and molecular biology.

PubMed Boolean search query

("Mesenchymal stem cells" [MeSH] or "Stem Cell, Mesenchymal" OR "Mesenchymal Stem Cell" OR "Stem Cells, Mesenchymal" OR "Mesenchymal Stromal Cells" OR "Mesenchymal Stromal Cell" OR "Stromal Cell, Mesenchymal" OR "Stromal Cells, Mesenchymal" OR "Multipotent Mesenchymal Stromal Cells" OR "Multipotent Mesenchymal Stromal Cell" OR "Mesenchymal Stromal Cells, Multipotent" OR "Mesenchymal Progenitor Cell" OR "Mesenchymal Progenitor Cells" OR "Progenitor Cell, Mesenchymal" OR "Progenitor Cells, Mesenchymal" OR "MSC") AND ("Secretome" [MeSH] OR "Secretomes" OR "Protein Secretome" OR "Protein Secretomes" OR "Secretome, Protein" OR "Culture Media, Conditioned" [MeSH] OR "Conditioned Medium" OR "Medium, Conditioned" OR "Culture Medium, Conditioned" OR "Conditioned Culture Media" OR "Conditioned Media" OR "Media, Conditioned" OR "Conditioned Culture Medium" OR "Extracellular vesicles" [MeSH] OR "Extracellular Vesicle" OR "Vesicle, Extracellular" OR "Vesicles, Extracellular" OR "Exovesicles" OR "Exovesicle" OR "Apoptotic Bodies" OR "Apoptotic Body" OR "Bodies, Apoptotic" OR "Body, Apoptotic" OR "Exosomes" OR "microvesicle" OR "microvesicles") AND ("Aging" [MeSH] OR "senescence" OR "biological aging" OR "aging, biological" OR "Cellular Senescence" [MeSH] OR "Senescence, Cellular" OR "Cell Senescence" OR "Senescence, Cell" OR "Cell Aging" OR "Cellular Ageing" OR "Ageing, Cellular" OR "Aging, Cell" OR "Senescence, Replicative" OR "Cellular Aging" OR "Aging, Cellular" OR "Replicative Senescence" OR "Cell Ageing" OR "Ageing, Cell").

Scopus Boolean search query

("Mesenchymal Stem Cell" OR "Mesenchymal Stromal Cell" OR "Multipotent Mesenchymal Stromal Cell" OR "Mesenchymal Progenitor Cell" OR "MSC") AND ("Secretome" OR "Protein Secretome" OR "Conditioned Medium" OR "Conditioned Culture Media" OR "Conditioned Media" OR "Conditioned Culture Medium" OR "Extracellular Vesicle" OR "Exovesicle" OR "Apoptotic Body" OR "Exosome" OR "microvesicle") AND ("Aging" OR "senescence" OR "biological aging" OR "Cellular Senescence" OR "Cell Senescence" OR "Cell Aging" OR "Cellular Ageing" OR "Cellular Aging" OR "Replicative Senescence" OR "Cell Ageing").

A total of 687 articles were identified across both platforms. After excluding duplicates, 307 articles underwent evaluation based on title and abstract. Among these, 39 articles were reviewed and analyzed. Studies not utilizing MSC-derived secretome or related terms for rejuvenation treatment were excluded. Additionally, articles reporting only *in vivo* results were removed from consideration.

Literature Search Results

Study characteristics

Figure 2 presents the details of the analyzed studies. Initially, the origin of MSCs and the types of secretome utilized in the rejuvenation strategy were assessed. Among the 39 articles reviewed, 29 obtained MSCs from humans, five from mice, four from rats, and one did not specify the species (Figure 2A). MSCs were isolated from various adult and fetal tissue sources. Adipose tissue was the most common source for adult MSC isolation (11 articles), followed by the umbilical cord (9 articles) (Figure 2C). Other sources included bone marrow (9 articles),

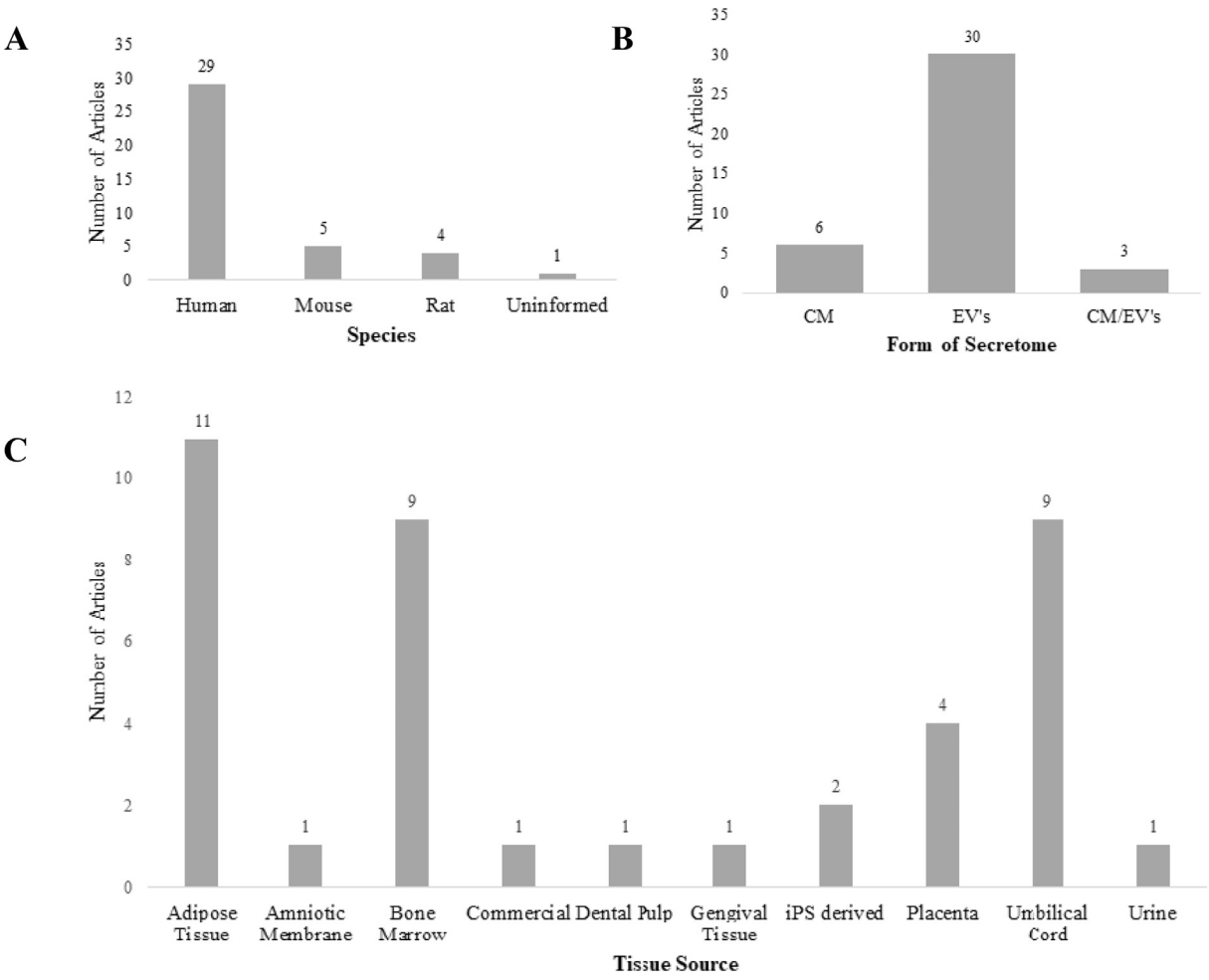

Fig. 2. *General information on the articles included in this review.* (**A**) Animal species of MSC. (**B**) The form of MSC-derived secretome that is used to treat senescent cells. (**C**) Tissue sources to obtain MSCs. CM: conditioned medium; EV: extracellular vesicle.

placenta (4 articles), gingival tissue (1 article), dental pulp (1 article), amniotic tissue (1 article), urine (1 article), and MSCs derived from induced pluripotent stem (iPS) cells (2 articles). One article did not specify the MSC source. Regarding the different forms of secretome, EVs (including exosomes) were the most frequently utilized (30 articles) (Figure 2B). Six articles utilized CM, three compared the effects of EVs and CM.

The studies reviewed, analyzed the effects of the MSC secretome on various cell types. Seven studies specifically examined the impact on MSCs derived from different human sources, including adipose tissue, bone marrow, dermal papillae, and umbilical cord (Table 1), which will be discussed in detail in the next section. Among the other cell types treated for rejuvenation (Table 2), dermal fibroblasts were the most extensively studied (ten articles), followed by keratinocytes (four articles), as well as endothelial and vascular cells (four articles). Additionally, connective tissue cells (six articles), cardiomyocytes (two articles), liver cells (one article), pancreatic cells (two articles), lymphocytes (one article), renal cells (one article), nervous system cells (two articles), and granulosa cells (one article) were also investigated.

The rejuvenating effects of MSC secretome on MSCs

Given that MSCs are extensively employed in cell-based tissue repair, evaluating their "fitness" and senescent state in culture is crucial. Consequently, strategies for their rejuvenation or expansion *in vitro* have been devised. Among the articles scrutinized in this review, seven assessed the application of MSC secretome to rescue or shield MSCs from senescence (Table 1). One study utilized CM, and six utilized EVs in the treatments. Despite variations in the tissue sources of MSCs and the methodologies employed to obtain the secretome, favorable outcomes were observed.

In this context, Wang *et al.* (2016) [38] demonstrated that CM derived from human fetal bone marrow mesenchymal stromal cells (BM-MSCs) could mitigate the replicative senescence of human adult BM-MSCs *in vitro* without inducing tumor formation *in vivo*. They observed a reduction in both the activity and expression of β-galactosidase, alongside enhanced proliferation characterized by increased numbers of cells in the S and G2/M phases, as well as enhanced osteogenic differentiation. Moreover, they noted restoration of the typical MSC morphology, accompanied by the downregulation of cell cycle inhibitors *p16* and *p53* (but upregulation of *p21*), and increased expression of histone deacetylase sirtuin 1 (*SIRT1*), which plays multiprotective roles against cellular senescence and stress.

EVs, which contain proteins, lipids, RNAs (such as miRNA, mRNA, and lncRNA), DNA fragments, and organelles, have emerged as a novel tool for MSC rejuvenation due to their ability to transfer genes with systemic effects and safety [19,39]. Liu *et al.* (2019) [40] demonstrated the positive impacts of EVs derived from human induced pluripotent stem cells (iPSCs) and human MSCs from various origins, including commercial suppliers, in alleviating senescent MSCs derived from different sources in both replicative and progerin-induced models of senescence. EVs from all sources increased cell proliferation, reduced β-galactosidase activity, downregulated the

Table 1
MSC-derived secretome treatment to rescue MSCs from senescence.

MSC-tissue source	The form of secretome tested	Cell treated for rejuvenation	Senescence assessed	Outcome	References
Immortalized human fetal BM-MSC cell line	Conditioned medium	Human adult BM-MSC	Replicative	↑ Proliferation ↑ S and G2/M phases ↑ Nonsenescent morphology No tumor formation ↑ Osteogenic differentiation ↓ β-galactosidase staining and activity ↑ *p21* mRNA ↓ *p16* mRNA ↓ *p53* mRNA ↓ *BAX* mRNA ↑ *SIRT1* mRNA	[38]
Human UC-MSC human iPSC	Extracellular vesicles	MSC (different sources)	Replicative (progerin-induced)	↓ β-galactosidase activity ↓ *p21* mRNA ↓ *p53* mRNA ↓ γ-H2AX ↓ IL-1α ↓ IL-6	[40]
Human infant AT-MSC	Extracellular vesicles	Aged human AT-MSC	Chronologic	↑ Proliferation ↓ β-galactosidase staining ↓ ROS ↑SOD1 protein ↑SOD3 protein ↑ERK protein	[41]
Adult human DP-MSC (low physiological O_2 tension)	Extracellular vesicles	Human adult DP-MSC	High O_2 tension	↓ β-galactosidase activity ↑ Proliferation ↑ Pluripotency factors ↓ Oxidative phosphorylation ↑ Glycolysis No change in ROS No change in the cell cycle ↑HIF-1α protein ↑*miR-302b*	[42]
Human UC-MSC	Extracellular vesicles	Human adult BM-MSC	Chronologic	Morphology (small cells) ↑ Proliferation ↑ Migration ↓β-galactosidase ↑ S phase cells ↑ CFU ↓ IL-6 ↓ IL-8 ↓ MCP-1 ↓ γ-H2AX ↑ Telomere length ↑*PCNA* mRNA ↑ Osteogenic differentiation ↓ Adipogenic differentiation No tumor formation	[43]
Human UC-MSC	Extracellular vesicles	Human aged BM-MSC	Chronologic	↑ Proliferation ↑ Migration ↑ S phase cells ↓ β-galactosidase staining ↓ p16 protein ↓ p21 protein ↓ p53 protein ↑SIRT1 protein ↑ *miR-136* ↓ *APAF1* mRNA ↓ Apoptosis	[44]
Human commercial lineage MSC	Extracellular vesicles	Human UC-MSC	Replicative	↑ Proliferation ↓ β-galactosidase staining and mRNA ↓ *p16* mRNA ↓ *IL1B* mRNA ↓ *IL6* mRNA	[45]

DP-MSC: dental Pulp-MSC; AT-MSC: adipose tissue-derived MSC; BM-MSC: bone marrow-derived MSC; β-galactosidase: β-galactosidase; CFU: colony-forming unit; DP-MSC: dental pulp-derived mesenchymal stromal cells; UC-MSC: umbilical cord-derived MSC; IL: interleukin; iPSC: induced pluripotent stem cells; MCP-1: monocyte chemotactic protein 1; ROS: reactive oxygen species; Sirt1: sirtuin 1.

expression of *p21* and *p53*, and mitigated DNA damage in senescent cells. Interestingly, EVs isolated from iPSCs exhibited a 16-fold increase compared to MSCs. Moreover, iPSC-EVs decreased ROS levels in progerin-induced senescent MSCs by delivering intracellular peroxiredoxin enzymes. In another study, EVs derived from infant adipose tissue MSCs (AT-MSCs) rejuvenated AT-MSCs from elderly donors by enhancing cell proliferation, reducing the number of β-galactosidase-positive cells, and inhibiting ROS elevation through

Table 2
MSC-derived secretome treatment to rescue different cell types from senescence.

MSC-tissue source	The form of secretome tested	Cell treated for rejuvenation	Method of senescence induction	Output	References
Human AT-MSC	Conditioned medium	Human dermal fibroblast HaCaT	UVB	↓ ROS ↓ IL-6 ↓ MMP1 mRNA and protein ↑ procollagen 1 mRNA ↓ pJNK protein ↓ pERK protein ↓ pp38 protein ↓ c-fos protein ↓ c-Jun protein ↓ NF$\kappa\beta$ protein ↓ HO-1 ↑ TGFβ ↑ Smad2/3 ↑ Smad7	[46]
Human AT-MSC	Extracellular vesicles	Human dermal fibroblast	H_2O_2	↑ Proliferation ↓ β-galactosidase staining ↓ ROS ↓ *p21* mRNA ↓ *p53* mRNA ↑ *SIRT1* mRNA ↑ Aquaporin 1 mRNA ↑Aquaporin 3 mRNA ↑ Hyaluronan secretion	[47]
Human AT-MSC	Conditioned medium	Human dermal fibroblast	H_2O_2	↑ Viability ↓ ROS ↑ COL protein	[48]
Human P-MSC	Extracellular vesicles	Human dermal fibroblast	High Glucose	↑ Proliferation ↑ Migration ↓ β-galactosidase staining ↑ S and G2/M phase cells ↓ ROS ↓ p21 protein ↓ RAGE protein ↑ COLI protein ↑ αSMA	[49]
P-MSC	Extracellular vesicles	Human dermal fibroblast	High Glucose	↑ Proliferation ↑ Migration ↓ β-galactosidase staining ↑ S and G2/M phase cells ↓ Apoptosis ↑ Bcl-2 mRNA and protein ↑ Cyclin D1 mRNA and protein ↓ Bax mRNA and protein ↑ *miR-145-5p*	[50]
Human UC-MSC	Extracellular vesicles	Human dermal fibroblast	UVB	↑ Proliferation ↓ β-galactosidase ↑ S phase cells ↓ ROS ↑ GPX1 protein ↑ COLI mRNA and protein ↓ MMP1 mRNA and protein	[52]
Rat UC-MSC	Conditioned medium	Rat dermal fibroblast	UVB	↓ β-galactosidase staining ↓ γH2AX ↓ p16 mRNA and protein ↓ p21 mRNA and protein ↓ p53 mRNA and protein ↓ *Il1a* mRNA ↓ *Il1b* mRNA ↓ *Il6* mRNA ↓ *Tnfa* mRNA ↑ LAMINB1 protein	[51]
Human BM-MSC	Extracellular vesicles	Human dermal fibroblast	UVB	↑ Viability ↑ Migration ↓ Apoptosis ↓ MDA ↑ SOD protein ↑ GPX protein ↑ *miR-29b-3p* ↑ *COLI* ↓ *MMP 1-2-3* mRNA	[53]

(*continued*)

Table 2 (*Continued*)

MSC-tissue source	The form of secretome tested	Cell treated for rejuvenation	Method of senescence induction	Output	References
Human G-MSC	Extracellular vesicles	Human dermal fibroblast HUVEC	H_2O_2	↑ Proliferation ↓ β-galactosidase staining ↓ γ-H2AX protein and foci ↓ p21 protein ↓ p53 protein ↓ mTOR/pS6 ↓ IL-6 secretion ↓ TNFα secretion ↓ pJNK ↓ p38MAPK ↑ eNOS (HUVEC) ↑ Tube formation (HUVEC)	[54]
Human AM-MSC	Conditioned medium	Human dermal fibroblast	H_2O_2	↑ Proliferation ↑ Migration ↓ β-galactosidase staining ↓ ROS ↓ γ-H2AX ↓ 8-OHdG ↓ p16 protein ↓ p21 protein and mRNA ↑ SOD ↑ Catalase ↓ MDA	[55]
Human UC-MSC	Extracellular vesicles	HaCaT	UVB	↑ Proliferation ↑ Migration ↓ Apoptosis ↑ Viability ↓ β-galactosidase staining ↓ ROS ↑ *COLI* mRNA ↓ *MMP1* mRNA	[56]
Human UC-MSC	Extracellular vesicles	HaCaT	UV/H_2O_2	↑ Proliferation ↓ Apoptosis ↓ ROS ↓ γ-H2AX ↓ 8-OHdG ↓ TNF-α secretion ↑ LC3II/I protein ↑ PCNA protein ↑14-3-3ζ protein ↑ Autophagy ↑ SIRT1 mRNA and protein ↑*Nrf2* mRNA	[57]
Human UC-MSC	Conditioned medium	HaCaT	UVB	↑ Nonsenescent morphology ↑ Proliferation ↑ Migration and motility ↑ Apoptosis ↓ G1 phase cells ↓ ROS ↓ p53 ↓ c-FOS protein ↓ c-JUN protein ↓ TGF-β protein ↑ Cyclin A2 protein ↑ FGF1 protein and mRNA ↓ *MYC* mRNA ↓ *CXCL8* mRNA ↓ *EREG* mRNA	[58]
Human AT-MSC	Extracellular vesicles	HUVEC	H_2O_2/high glucose	↑ Proliferation ↑ Nonsenescent morphology ↑ Migration ↑ Tube formation ↓ β-galactosidase staining ↓ p16 protein and mRNA ↓ p21 protein and mRNA ↓ p53 protein and mRNA ↑ LMNB1 protein and mRNA ↓ IL-6 secretion ↓ IL-8 secretion ↓ ROS ↑ Respiration capacity ↑ O_2 Consumption rate ↑*miR-146a* ↓pScr	[59]

(*continued*)

Table 2 (*Continued*)

MSC-tissue source	The form of secretome tested	Cell treated for rejuvenation	Method of senescence induction	Output	References
				↓ pVE-cadherin ↓p-caveolin1	
Mouse AT-MSC	Extracellular vesicles	Mouse endothelial progenitor cell	Older donors	↑ Proliferation ↓ β-galactosidase staining ↓ p16 protein ↓ *p19* mRNA ↑*miR-126* ↓*Spred 1* mRNA ↑ Tube formation/agiogenesis	[60]
Human AT-MSC	Extracellular vesicles	Human corneal endothelial cells (CEC)	H_2O_2 /TGF-β1	↑ Viability/proliferation ↑ S phase cells ↑ Migration ↓ β-galactosidase staining ↓ Autophagosome ↓ LC3II/LC3I rate ↓ N-cadherin protein ↑ Mitochondria membrane potential ↓ Mitochondrial oxidative stress ↑ pYAP protein ↓ Nuclear YAP ↑ *miR-302-3p* ↑ *miR-23a-3p*	[61]
Human AT-MSC	Extracellular vesicles	Mouse vascular smooth muscle cells (VSMC)	Angiotensin II	↑ Proliferation ↓ β-galactosidase staining ↓ p16 protein ↓ p21 protein ↓ mtROS ↓ Mitochondrial fission ↓ MMP-9 ↓ *IL6* mRNA ↓ *Ccl2* mRNA ↓ *Mmp2* mRNA ↑ *miR-19b-3p* ↓ MST4 protein ↓ pERK protein ↓ pDrp1 protein	[62]
Human iPSC-derived MSC	Extracellular vesicles	Human nucleus polposus cells	TNF-α	↑ Proliferation ↓ β-galactosidase staining ↓ p16 protein ↑ *miR-105-5p* ↑ SIRT6 protein ↓ PDE4D protein ↑ COL2 protein ↑ Aggrecan protein ↓ MMP-3 protein ↓ ADAMTS-4 protein	[63]
Rat BM-MSC	Conditioned medium Extracellular vesicles	Human nucleus polposus cells	H_2O_2	↑ Proliferation ↑ Viability ↑ Nonsenescent morphology ↓ β-galactosidase staining ↓ p16 protein ↓ p21 protein ↑ COL2 protein ↓ MMP13 secretion	[64]
Rat BM-MSC	Extracellular vesicles	Rat nucleus pulposus cells	Hypoxia	↓mtROS ↓ Mitochondrial fragmentation ↑ Mitochondrial polarization/área ↓ p16 protein ↓ p21 protein ↑ COL2 protein ↓ MMP13 protein ↓ IL-1β secretion ↓ IL-6 secretion	[65]
Human AT-MSC	Conditioned medium extracellular vesicles	Human osteoblast	IL-1β	↓ β-galactosidase activity ↓ γ-H2AX ↓ IL-6 secretion ↓ PGE2 secretion ↓ TNF-α secretion (CM only) ↑ IL-10 secretion ↓ HNA-protein adducts ↑ Mitochondria Membrane Potential	[66]

(*continued*)

Table 2 (*Continued*)

MSC-tissue source	The form of secretome tested	Cell treated for rejuvenation	Method of senescence induction	Output	References
Human UC-MSC	Extracellular vesicles	Human OA chondrocyte	Osteoarthritis	↑ Nonsenescent morphology ↑ Proliferation ↓ β-galactosidase staining ↓ *p16* ↓ *p21* ↓ p53 mRNA and protein ↑ *LaminB1* mRNA ↓ *IL1* mRNA ↓ *IL8* mRNA ↓ *NFKB* mRNA ↓ *MMP13* mRNA ↑ COL2 mRNA and protein ↓ COL1A *m*RNA and protein ↑ *SOX9* mRNA ↑ *ACAN* mRNA ↓ *COL10A m*RNA ↑ GAG	[67]
Mouse AT-MSC	Extracellular vesicles	Mouse tenocytes/ macrophages	IL-1β /TGF-β1	**Tenocytes/IL-1β:** ↑ Viability ↓ Apoptosis ↑ Migration ↓ β-galactosidase staining ↓ p16 protein ↓ p21 protein ↑ SIRT1 protein ↑ NAMPT protein ↑ NAD^+ production ↑ Mitochondrial functions **Tenocytes/ TGF-β1**: ↓ α-SMA protein ↓ Collagen protein ↓ *COL1* mRNA ↓ *COL3* mRNA ↓ *MMP1* mRNA ↓ *MMP3* mRNA ↓ *TIMP1* mRNA **Macrophages/ IL-1β:** ↑ Phagocytosis ↓ p16 protein ↓ p21 protein ↑ NAMPT protein ↑ NAD^+ production ↑ M2 macrophages ↑ M2 markers ↓ IL6 secretion ↓ IL8 secretion ↓ MCP1 secretion ↓ MMP3 secretion ↑ TGF-β1 secretion ↑ SIRT1 protein ↓ p65 protein ↓ NLRP3 protein ↓ ASC protein	[69]
Human AT-MSC	Extracellular vesicles	Human PSC-derived cardiomyocyte	Doxorubicin	↓ β-galactosidase staining ↓ G0/G1 phase cells ↓ p21 mRNA and protein ↓ p53 mRNA and protein ↑ *LncRNA-MALAT1* ↓ *miR-92-3p* ↑ ATG4a protein	[69]
Mouse BM-MSC	Extracellular vesicles	Mouse cardiomyocyte	Doxorubicin	↓ β-galactosidase staining ↓ G1 phase cells ↓ *p16* mRNA ↓ *p27* mRNA ↑ Telomere length ↑ Telomerase activity ↑ *LncRNA-NEAT1* ↓ *miR-221-3p* ↑ SIRT2 mRNA and protein	[70]

(*continued*)

Table 2 (*Continued*)

MSC-tissue source	The form of secretome tested	Cell treated for rejuvenation	Method of senescence induction	Output	References
Human UC-MSC	Conditioned medium Extracellular vesicles	Hepatocytes	H_2O_2	↑ Proliferation (EV only) ↓ β-galactosidase staining ↓ p16 protein ↓ p21 protein ↑ PCNA protein ↓ *IL1A* mRNA ↓ *IL1B* mRNA ↓ *IL6* mRNA ↓ *IL8* mRNA ↓ *CCL20* mRNA ↑ Mitophagy ↓ Mitochondrial damage ↑ Atg4B protein	[73]
Mouse BM-MSC	Extracellular vesicles	Mouse βTC-6 cells	Hypoxia	↑ Proliferation ↓ β-galactosidase staining ↓ p16 protein ↓ p21 protein ↓ p53 protein ↑ *Cdk2* mRNA ↑ *Cdk6* mRNA ↑ Cyclin D1 mRNA ↓ Apoptosis ↑ Autophagy ↑ LC3BII/I ratio ↓ SQSTM1 protein ↑ YTHDF1 protein	[72]
Human P-MSC	Extracellular vesicles	Mouse cholangioids	H_2O_2	↓ β-galactosidase ↑ Proliferation ↓ p16 protein ↓ p21 protein ↓ IL6 mRNA and secretion ↓ CCL2 mRNA and secretion ↓ CXCL1 mRNA and secretion ↓ *Cxcl2* mRNA ↓ *Cxcl9* mRNA ↓ *Cxcl16* mRNA ↓ *Cx3cl1* mRNA ↓ CXCL10 secretion	[71]
Human P-MSC	Extracellular vesicles	Human $CD4^+$ T cells	D-galactose	↓ β-galactosidase staining ↓ p53 protein ↓ γ-H2AX protein ↓ 8-OHdG ↓ ROS ↑ SOD activity ↓ *IL6* mRNA ↓ *OPN* mRNA ↑ *miR-21* ↓ PTEN protein ↑ Nuclear Nrf2 protein ↑ HO-1 protein ↑ NQO1 protein	[75]
Human commercial lineage MSC	Extracellular vesicles	Mouse primary tubular Epithelial cells	Radiation	↑ Proliferation ↓ γ-H2AX marker ↓ *Cdkn2a* mRNA ↓ *Cdkn2d* mRNA ↑ *Lmb1* mRNA ↓ *Il6* mRNA ↓ *Ccl7* mRNA	[45]
MSC	Extracellular vesicles	Mouse brain/microglial cells (BV-2)	H_2O_2	↑ Proliferation ↑ Migration ↓ Apoptosis ↓ β-galactosidase staining ↓ ROS ↑ GSH-Px ↑ T-SOD ↑ Bcl2 protein ↓ Bax protein ↑ SIRT1 protein ↓ p21 protein ↓ p53 protein	[77]

(*continued*)

Table 2 (*Continued*)

MSC-tissue source	The form of secretome tested	Cell treated for rejuvenation	Method of senescence induction	Output	References
Human U-MSC	Extracellular vesicles	Retinal ganglion cells (RGC)	D-Galactose	↑ Proliferation ↑ Viability ↓ Apoptosis ↓ β-galactosidase staining	[74]
Rat BM-MSC	Extracellular vesicles	Human granulosa cells	H_2O_2	↓ β-galactosidase staining ↓ ROS ↓ p21 protein ↑ *circLRRC8A* ↓ *miR-125a-3p* ↑ NFE2L1 mRNA and protein Restoration of estrous cycle	[76]

AM-MSC: amniotic membrane tissue-derived MSC; AT-MSC: adipose tissue-derived MSC; BM-MSC: bone marrow-derived MSC; β-galactosidase: β-galactosidaseactosidase; G-MSC: gengival tissue MSC tissue-derived MSC; HaCaT: high sensitivity of human epidermal keratinocytes; HNA: 2-hydroxy-1-naphthaldehyde; HUVEC: human umbilical vein endothelial cells; IL: interleukin; iPSC: induced pluripotent stem cells; P-MSC: placenta MSC; PGE2: prostaglandin E2; ROS: reactive oxygen species; TNF-α: tumor necrosis factor alpha; U-MSC: urine-derived MSC; UC-MSC: umbilical cord-derived MSC.

upregulation of antioxidant enzymes superoxide dismutase (SOD)1 and SOD 3 [41]. Additionally, Mas-Bargues *et al.* (2020) [42] demonstrated the beneficial effects of EVs from nonsenescent human adult dental pulp-derived MSCs (DP-MSCs) maintained under low physiological oxygen tension in rescuing prematurely senescent DP-MSCs cultured in a hyperoxic environment. This treatment resulted in reduced β-galactosidase activity, enhanced cell proliferation, overexpression of pluripotency factors (*OCT4, SOX2, KLF4,* and *cMYC* or OSKM), increased glycolysis, and reduced oxidative phosphorylation. The authors suggested that this effect was mediated by the upregulation of *miR-302b* and hypoxia-inducible factor-1α (HIF-1α) levels in the target cells. Similarly, Lei *et al.* (2021) [43] reported that EVs from umbilical cord-derived MSCs (UC-MSCs) were able to rejuvenate senescent BM-MSCs from adult humans, alleviating aging phenotypes and increasing self-renewal capacity and telomere length through the transfer of proliferating cell nuclear antigen (*PCNA*). Intriguingly, intravenous injection of UC-MSC-derived EVs, originating from a youthful MSC source, reduced bone and kidney degeneration in aged mice and improved skin wound repair.

In addition, Zhang *et al.* (2020) [44] demonstrated that EVs derived from human UC-MSCs ameliorated the senescent phenotype of BM-MSCs from elderly donors by promoting cell proliferation and reducing apoptosis rates. They observed a decrease in the number of β-galactosidase-positive cells and reduced expression of p53, p21, and p16 proteins. The authors further illustrated that rejuvenated elderly BM-MSCs exhibited enhanced activity in cardiac repair, characterized by increased neovascularization and reduced scar formation, mediated by EV *miRNA-136* and the downregulation of its direct target apoptotic peptidase activating factor 1 (APAF1), a critical regulator of cell survival and apoptosis [44]. Similarly, Liao *et al.* (2021) [45] demonstrated the proproliferative and antisenescent effects of EVs derived from human MSCs on replicative senescent UC-MSCs. They observed reductions in β-galactosidase-positive cells. Additionally, they found decreased expression of *p16* mRNA, increased Ki67 positivity, and reduced expression of the proinflammatory cytokines genes *IL6* and *IL1B,* suggesting attenuation of SASP-related activity.

Rejuvenating effects of MSC secretome on various cell types

The 33 articles assessed demonstrated the rejuvenating potential of MSC-derived secretomes from multiple tissue sources and formulations on cell types beyond MSCs. These findings are outlined in Table 2.

Skin Cells: Dermal Fibroblasts and Keratinocytes

Dermal fibroblasts were the most frequently utilized cell type for studying the rejuvenating effects of MSC secretome, followed by the human adult skin keratinocyte lineage HaCaT. Ten studies investigated the effects of CM, and/or EVs obtained from human AT-MSCs [46–48], placenta-derived MSCs (P-MSCs) [49,50], UC-MSCs [51,52], BM-MSCs [53], gingival tissue-derived MSCs (G-MSCs) [54], and amniotic tissue-derived MSCs (AM-MSCs) [55] on dermal fibroblasts. Four studies examined the effects of CM or EVs from human AT-MSCs [46] or human UC-MSCs [56–58] on HaCaT senescence induced by UVB radiation or H_2O_2.

Fibroblast senescence was induced through various methods, including exposure to UVB irradiation [46,51–53], H_2O_2 [47,48,54,55], or high glucose concentrations [49,50]. Overall, the MSC secretome stimulated cell viability, proliferation, and migration, facilitating re-entry into the cell cycle as evidenced by the increased number of cells in the S phase, decreased expression of p21, p16, and p53, and decreased β-galactosidase activity. Additionally, articles reported the restoration of extracellular matrix production and remodulation by increasing collagen type I (COL I) levels and reducing matrix metalloproteinase (MMP) levels. Improved antioxidant defenses were also observed, as evidenced by reduced intracellular ROS, accompanied by a reduction in DNA damage markers (γ-H2AX and 8-OHdG) and suppression of SASP production [46–55].

More specifically, Li and colleagues' study (2019) [45] revealed the photoprotective effect of CM from AT-MSCs against UVB radiation by reducing IL-6 secretion and regulating ROS levels. This result involved the modulation of responsive signaling pathways, including mitogen-activated protein kinases (MAPKs), activator protein 1 (AP-1), nuclear factor kappa B (NF-κB), as well as the TGF-β/Smad pathway, resulting in reduced MMP1 expression and increased procollagen-1 mRNA expression. Moreover, EVs from AT-MSC were able to protect against the oxidative stress induced by H_2O_2 mediated by the upregulation of *SIRT1* [47]. Similarly, Chen *et al.* (2023) [51] observed that CM from rat UC-MSCs alleviated the *in vivo* progression of skin ulcers induced by UVB radiation in rats, accompanied by decreased serum levels of IL-1α. Additionally, UVB-induced skin fibroblast photoaging was mitigated by treatment with EVs derived from human BM-MSCs. This effect was mediated by *miR-29b-3p* and accompanied by a decrease in the oxidative stress marker malondialdehyde (MDA) and an increase in SOD and glutathione peroxidase (GPX) enzymes [53].

Also, Shi *et al.* (2021) [54] discovered that EVs derived from G-MSCs were capable of mitigating H_2O_2-induced senescence in human dermal fibroblasts by modulating the mTOR signaling network, which plays a central role in aging. They also demonstrated that systemic administration of EVs attenuated elevated levels of p21, mTOR/pS6, IL-6, and TNF-α in the skin and heart of aged mice. Additionally, CM from human AM-MSCs delayed H_2O_2-induced senescence in human dermal fibroblasts by promoting an increase in SOD and catalase activity while decreasing MDA, thereby reducing ROS production [55]. Moreover, EVs from P-MSCs enhanced wound healing in

diabetic mice by suppressing the RAGE pathway, activating the Smad pathway [49], or transferring *miR-145-5p*, which activates the Erk/Akt signaling pathway [50], thus improving high glucose-induced senescent fibroblasts.

CM from AT-MSCs [46] and EVs and CM from UC-MSCs [56–58] were also investigated for their effects on UV/UVB-induced photoaging in the human adult skin keratinocyte lineage HaCaT. The rejuvenating effects were associated with increased proliferation and migration and reduced apoptosis, as evidenced by decreased numbers of cells in the G1 phase, restoration of cell morphology, decline in p53 and intracellular levels of ROS, β-galactosidase activity, and the DNA damage markers γ-H2AX and 8-OHdG [46,56–58]. Treatment with MSC-derived EVs led to a decrease in TNF-α levels while increasing those of *SIRT1* and *Nrf2* associated with LC3II/I [57]. The authors suggest that the 14-3-3ζ protein delivered by UC-MSC EVs exerts a cytoprotective function via the modulation of a SIRT1-dependent antioxidant pathway. Regarding senescent dermal fibroblasts, Li *et al.* (2019) [46] demonstrated that the effects of CM from AT-MSCs in preventing HaCaT senescence involve the reduction of c-FOS, c-JUN, and JNK, ERK, and p38 phosphorylated forms. Moreover, Zou *et al.* (2022) [58] demonstrated, through multiomics analysis, that the antiphotoaging-UVB effects of human UC-MSCs-CM on HaCaT involve the key genes *MYC, IL8, FGF1*, and *EREG*, and the key proteins c-FOS, c-JUN, TGFβ, p53, FGF-1, and cyclin A2.

Vessel Cells

Human G-MSC-EVs and AT-MSC-EVs stimulated cell proliferation and migration, restoring tube formation in H_2O_2-senescent human umbilical vein endothelial cells (HUVECs), accompanied by the recovery of cellular and nuclear morphology. Reduced activity of β-galactosidase, decreased expression of γ-H2AX, p16, p21, and p53, as well as increased expression of the nuclear matrix protein Lamin B1 and endothelial nitric oxide synthase (eNOS), were observed. Additionally, there was a reduction in the levels of ROS and the SASP factors IL-6 and IL-8 [54,59]. Mechanistically, the effects of G-MSC-EVs involve the mTOR/S6 pathway [54]. In contrast, those of AT-MSC-EVs involve the *miR-146a/Src* signaling pathway, which was also capable of promoting wound closure and new blood vessel formation in aged and type-2 diabetes mice [59].

Furthermore, EVs derived from young, but not aged, mouse AT-MSCs were able to rejuvenate senescent mouse endothelial progenitor cells (EPCs) *in vitro* by reducing β-galactosidase activity and the expression of p16 while also promoting angiogenesis *in vivo*. These effects were mediated by *miR-126*, resulting in the reduction of SPRED-1, an inhibitor of the vascular endothelial growth factor (VEGF) pathway [60]. This finding suggests the activation of the VEGF pathway to enhance the tube formation capacity and proliferation rate of EPCs.

Additionally, human AT-MSC-EVs were shown to alleviate H_2O_2- or TGF-β1-induced oxidative stress and senescence in human Corneal Endothelial Cells (CECs) by enhancing cell viability and proliferation, suppressing autophagy, reducing β-galactosidase staining, and mitigating mitochondrial oxidative stress. In an *in vivo* approach, they facilitated wound healing of rat CECs and shielded the corneal endothelium from cryoinjury-induced damage by involving *miR-302-3p* and *miR-23a-3p*, as well as the HIPPO-YAP pathway [61]. Human AT-MSC-EVs also attenuated angiotensin II-induced senescence in mouse vascular smooth muscle cells (VSMCs) by inhibiting mitochondrial fission through *miR-19b-3p*-mediated downregulation of the MST4/ERK/Drp1 signaling pathway. This led to increased cell proliferation, reduced expression of cell cycle inhibitors p16 and p21, decreased β-galactosidase staining, and lowered mitochondrial ROS levels both *in vitro* and *in vivo* in a mouse model of angiotensin II-induced abdominal aortic aneurysm. Furthermore, AT-MSC-EVs treatment mitigated aortic wall dilation and thickness, accompanied by a decrease in CD68 macrophages and MMP9 levels in aortic tissue [62].

Connective Tissues

The MSC secretome has also been investigated as a potential therapeutic strategy for intervertebral disc degeneration. In this context, CM and EVs derived from human iPSC-derived MSCs [63] and rat BM-derived MSCs [64,65] were found to reverse the senescent phenotype of human and rat nucleus pulposus cells (NPCs) induced by tumor necrosis factor α (TNF-α), H_2O_2, or hypoxia. These various forms of MSC secretome were able to mitigate NPC senescence by restoring cell viability and proliferation, reducing expression of p16 and p21, as well as β-galactosidase staining. Additionally, they alleviated oxidative stress and mitochondrial damage and promoted extracellular matrix deposition *in vitro*. Moreover, in a rat *in vivo* model, they demonstrated the ability to attenuate intervertebral disc degeneration [63–65]. A reduction in the SASP factors IL-1β and IL-6 accompanied these effects [65]. The mechanism involves the activation of the SIRT-6 pathway and *miR-105-5p*, which downregulates the cAMP-specific hydrolase PDE4D [63].

Moreover, the MSC secretome has been explored for the treatment of osteoarthritis. Tofiño-Vian *et al.* (2017) [66] demonstrated that CM or EVs derived from human AT-MSCs stimulated by IL-1β downregulated β-galactosidase activity, reduced γ-H2AX foci and decreased the levels of the proinflammatory mediators IL-6 and prostaglandin E2 in osteoarthritic osteoblasts. The senescent phenotype was attenuated by treatment with EVs derived from human UC-MSCs engineered to target chondrocytes, resulting in the restoration of nonsenescent morphology, increased cell proliferation, and upregulation of Lamin B1 expression, accompanied by a decrease in the expression of p16, p21, and p53, β-galactosidase staining, and release of SASP factors. Moreover, the restoration of the osteoarthritic cartilage health phenotype was observed in an osteoarthritic rat model [67].

In vitro and *in vivo* studies conducted by Wu *et al.* (2023) [68] revealed the protective effects of EVs derived from AT-MSC-EVs obtained from young mice, but not from older ones, against tendinopathy, an age-related degenerative disorder. The mechanism involves the improvement of viability, migration, and mitochondrial metabolism in IL-1β- and TGF-β1-induced senescent tenocytes, accompanied by a reduction in β-galactosidase staining, as well as decreased levels of p16, p21, α-smooth muscle actin (αSMA), COL I, COL II, MMP1, MMP3, and tissue inhibitor of MMP1 (TIMP1) through the NAMPT/SIRT1/PPARγ/PGC-1α pathway. Furthermore, the authors reported enhanced phagocytosis and polarization of M2 macrophages through the NAMPT/SIRT1/NF-κB p65/NLRP3 pathway.

Cardiomyocytes

Cardiomyocytes represent another cell type rejuvenated by MSC EVs. In this regard, EVs derived from human AT-MSCs cultured under hypoxic conditions [69] and from mouse BM-MSCs pretreated with macrophage migration inhibitory factor (MIF) [70] have been demonstrated to rescue doxorubicin-induced senescent cardiomyocytes. This rescue is characterized by a reduction in the number of cells in the G0/G1 phase, decreased β-galactosidase activity, and downregulation of the expression of p21, p53, p16, and p27 while maintaining telomere length and telomerase activity. The rejuvenating effect of hypoxia-preconditioned AT-MSC EVs is attributed to the long noncoding RNA (LncRNA) *MALAT1*, which inhibits *miR-92a-3p* and activates ATG4a (Xia *et al.*, 2020), whereas that of MIF-preconditioned BM-MSC EVs occurs through the transfer of *LncRNA-NEAT1*, which inhibits *miR-221-3p* and leads to SIRT2 activation [70].

Liver and Pancreatic Cells

The MSC secretome has also been shown to rescue liver and pancreatic cells from senescence induced by H_2O_2 and hypoxia [71–73]. Human UC-MSC EVs were demonstrated, through *in vitro* experiments, to enhance the proliferation and restore the mitochondrial function of H_2O_2-induced senescent hepatocytes by upregulating mitophagy and reducing β-galactosidase staining, as well as the expression of p16 and p21 and the expression of SASP factors. This mechanism involves the transfer of DEAD-box helicase 5 (DDX5) enriched in MSC-EVs to aged hepatocytes, promoting the nuclear translocation of E2F transcription factor 1 (E2F1), followed by the upregulation of ATG4B expression. Furthermore, *in vivo* experiments conducted in an aged mice model of partial hepatectomy confirmed the role of EV treatment in liver regeneration [73].

Additionally, Fang *et al.* (2022) [72], studying the pancreatic tumor lineage βTC-6, demonstrated the effects of HIF-1α overexpressed in EVs derived from mouse BM-MSCs in ameliorating hypoxia-induced β-cell death during islet transplantation. The treatment was able to restore the senescent phenotype, increasing cell proliferation and reducing apoptosis, accompanied by a decrease in β-galactosidase activity and the expression of p16, p21, and p53. Mechanistically, BM-MSC-derived HIF-1α-enriched EVs activated YTHDF1-mediated protective autophagy by upregulating autophagy-associated proteins (ATG5, ATG2A, and ATG14).

Moreover, human P-MSC-derived EVs were shown to delay the aging process in senescent H_2O_2-induced organoids of cholangiocytes, the epithelial cells of the bile duct. This delay was achieved through the negative regulation of p21 and p16, resulting in enhanced proliferation. Treatment also reduced β-galactosidase positivity and expression of the SASP components IL-6, CCL2, CXCL1 [71].

Other Cell Types

In addition to the cell types mentioned above, EVs derived from MSCs (including human P-MSCs, a commercially available human MSC, human UC-MSCs, and rat BM-MSCs) were capable of rescuing CD4+ T lymphocytes, primary kidney epithelial cells (PTECs), a microglial lineage (BV-2), retinal ganglion cells (RGCs), and granulosa cells from senescence induced by D-galactose [74,75], radiation [45], or H_2O_2 [76,77]. Generally, the MSC secretome stimulates cell proliferation and migration by reducing the expression of negative cell cycle regulators such as p53, p21, p16, or p19. Articles also reported reduced apoptosis observed by decreased β-galactosidase staining, improved antioxidant defenses by reducing intracellular ROS, accompanied by a reduction in DNA damage markers such as γ-H2AX and 8-OHdG, and reduced production of SASP factors including IL-6, OPN, and CCL-6 [45,74–77].

Specifically, the study by Xiong *et al.* (2021) [75] revealed that the protective effect of EVs from human P-MSCs in attenuating D-galactose-induced senescence in CD4+ T cells involve the transfer of *miRNA-21* and activation of the PTEN/PI3K-Nrf2 pathway. Additionally, MSC EVs were effective in safeguarding BV-2 cells from H_2O_2 oxidative damage, thereby delaying brain aging through modulation of the SIRT1/p53 pathway [77]. Through bioinformatics analysis and functional assays, Xing *et al.* (2023) [76] demonstrated that *circLRRC8A* plays a pivotal role in the EVs of rat BM-MSCs in protecting against oxidative damage and counteracting senescence in granulosa cells, both *in vitro* and *in vivo*, via the *miR-125a-3p*/NFE2L1 axis. *In vitro* experiments indicated the attenuation of H_2O_2-induced senescence in a human granulosa cell lineage through this treatment, leading to reductions in β-galactosidase staining and ROS production, as well as the restoration of estrous cycle balance in a premature ovarian failure rat model. The authors propose a novel role for *circLRRC8A* in preventing granulosa cell senescence by functioning as a *miR-125a-3p* sponge, ultimately resulting in the upregulation of NFE2L1, a critical factor in regulating the adaptive antioxidant response to oxidative stress.

Discussion

The present article highlights the effects of the MSC secretome on cell rejuvenation. First, the authors included background information regarding the characteristics of the senescent phenotype that are utilized as biomarkers. This information is critical to understanding the use of the MSC secretome as an antisenescent strategy. Next, the authors chose articles regarding the *in vitro* approach with the use of CM or EVs from MSCs to recover cell senescence based on a quasi-systematic investigation. Additionally, beyond showing a list of tissue sources of MSC-derived secretome and the cells treated for rejuvenation, the authors provided information concerning the types of senescence assessed and the outcomes and mechanisms involved.

It should be noted that the authors focused on the cellular aspects of senescence evaluated *in vitro*. Therefore, only articles that employed cell culture analysis were included, although some *in vivo* data were mentioned to complement the *in vitro* results. The authors consider these key aspects to be of high importance to the field, particularly in the development of protocols for preclinical and clinical studies. Following the recommendations of the International Society for Extracellular Vesicles (ISEV), the term "extracellular vesicle" was used as a generic descriptor for "particles released from cells that are enclosed by a lipid bilayer and cannot self-replicate" [78,79]. Consequently, articles referencing both "extracellular vesicles" and "exosomes" were grouped in the analysis.

The primary discovery of this study is the demonstration that irrespective of the form (CM or EV), animal species (human, mice, or rat), and tissue origin (bone marrow, umbilical cord, adipose tissue, dermal papillae, gingiva, amniotic tissue, and placenta), the secretome of MSCs was capable of rescuing cells from a broad spectrum of senescence, including replicative, chronological, or induced by different stimuli such as UVB radiation, H_2O_2, TNF-α, hypoxia, high glucose concentration, or doxorubicin. The results reinforce the diversity of senescent phenotypes (from humans, mice, or rats) and disease or injury models that MSC-derived secretome could modulate. Dermal fibroblasts were the most investigated cell type, followed by MSCs (derived from adipose tissue, bone marrow, dermal papillae, and umbilical cord), keratinocytes (HaCaT lineage), HUVEC, connective tissue cells, cardiomyocytes, liver and pancreatic cells, and immune, renal, nerve, and granulosa cells. MSC-derived secretome have been investigated to treat wound healing, skin ulcers, aneurysms, intervertebral disc degeneration, osteoarthritis, tendinopathy, and chronological senescence.

Figure 3 summarizes the cellular characteristics modulated by the MSC secretome and considered as biomarkers. Investigations have shown recovery of cell morphology, nuclear architecture (increased expression of the LMNB1 gene), and production and remodulation of the extracellular matrix (increased COL I production and reduction of MMP1 or MMP9). It also observed improved cell migration, viability, and proliferation (increasing cell number, PCNA staining, S phase cell number and reducing G1 phase cell number, and p16, p53, p27, and p21 cell cycle inhibitors), attenuation of apoptosis, reducing β-galactosidase staining, increased autophagy, and recovery of mitochondrial functions (reducing mitophagy and mitochondrial stress and increasing glycolysis). MSC-derived secretomes were investigated to treat wound healing, skin ulcers, aneurysms, intervertebral disc degeneration, osteoarthritis, tendinopathy, and chronological senescence. In addition, the results revealed control of oxidative stress (reduction of ROS by increasing the expression of the antioxidant enzyme), reduction of DNA damage (decrease in H2AX foci, expression of 8-OHdG and increase in telomerase activity and telomere length), and epigenetic changes (increase in SIRT1 and Nrf2 levels).

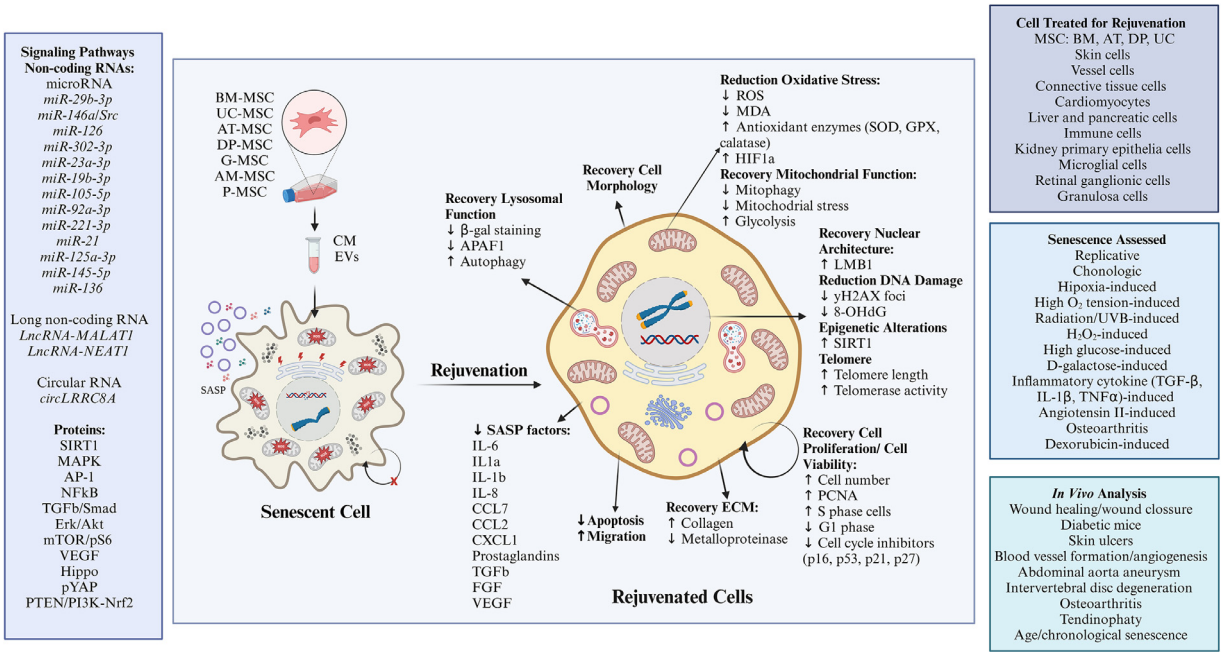

Fig. 3. Cell rejuvenation by MSC-derived secretome. Secretome of MSC (different sources) treatment was able to recover alterations in senescent cells (different types) such as cell morphology, reduction in oxidative stress and recovery of mitochondrial function, recovery of lysosomal function, reduction of DNA damage and recovery of nuclear architecture, increase cell proliferation, viability and migration and decrease apoptosis and SASP. Proteins and noncoding RNAs are involved in signaling pathways of cell regeneration. In vivo analyses were also evaluated, such as wound healing, osteoarthritis, intervertebral disc degeneration, and aging, among others. Created with BioRender.com. (Color version of figure is available online.)

The results also revealed a reduction in the SASP factors IL-1, IL-1, IL-6, IL-8, CCL-7, CCL2, CXCL1, Prostaglandins, TGB, FGF, and VEGF.

It is noteworthy that the method of cell treatment for rejuvenation varies according to the type of senescence under investigation. Studies focusing on MSC rejuvenation have primarily examined chronological aging [38,41,43,44] or replicative senescence [40,42,45]. This approach is crucial, given the time required for MSC expansion in culture and the age of MSC donors, both of which present limitations for MSC therapy. Consequently, utilizing the secretome of MSCs from young donors during MSC growth and expansion for therapy holds promise. Conversely, most studies investigating rejuvenation in cells other than MSCs have examined senescence induced by external stressors such as radiation and H_2O_2. In addition, the senescent phenotype detected varied depending on the stress stimuli to which the cells were exposed. Therefore, employing multiple senescence biomarkers simultaneously and conducting a comprehensive set of analyses are essential for accurately assessing antisenescence strategies. The method for processing the secretome should also be carefully chosen since differences in results using CM or EVs were observed.

Moreover, it should be noted that senescence is a crucial process in maintaining healthy physiology, playing essential roles in tissue repair and embryogenesis. It also acts as a protective mechanism by preventing the proliferation of damaged cells through irreversible cell cycle arrest, thereby serving as a critical “check and balance” against malignant transformation [10,80]. This role is complex and somewhat paradoxical: while senescence can inhibit tumorigenesis, it can also contribute to tumor progression via the senescence-associated secretory phenotype (SASP) [80]. Thus, studies on reversing senescence should be approached with caution and precision, considering the risk of hyperplastic pathology. Therefore, it is essential to develop strategies that minimize these risks to ensure the safety of potential therapeutic applications.

It is essential to consider the molecular and structural heterogeneity of EVs, as this reflects the physiological state of the producing cells and can influence the function and phenotype of recipient cells [79]. Cell culture conditions, such as cell type, viability, passage number, and seeding density, directly and indirectly impact the yield, composition, and production of EVs. In response to these complexities, the ISEV recently updated its guidelines, the "Minimal Information for Studies of Extracellular Vesicles" (MISEV2023), which provide an in-depth overview of methods for EV production, isolation, and characterization, outlining their benefits and limitations [79]. The diversity of isolation and separation techniques, which rely on different mechanisms, further contributes to variations in EV populations. Additionally, factors like sample collection, preprocessing, and storage conditions affect EV quantity and quality, highlighting the importance of donor characteristics and the availability and quality of source material. The lack of standardization in preprocessing adds further complexity. Although this review did not distinguish between types of EVs used in rejuvenation strategies, it is crucial to follow MISEV2023 guidelines for accurate classification, source identification, and comprehensive descriptions of isolation and characterization techniques. This will enhance methodological transparency and improve the reproducibility of future studies.

Mechanically, the articles analyzed reported the involvement of several signaling pathways. The upregulation of SIRT1 was the most cited, but the MAPK, AP-1, NF, TGF/Smad, Erk/Akt, mTOR/pS6, VEGF, HIPPO, pYAP or PTEN/PI3K-Nrf2 pathways were also reported, as well as noncoding RNAs (*miR-29b-3p, miR-146a/Src, miR-126, miR-302-3p, miR-23a-3p, miR-19b-3p, miR-105-5p, miR-92a-3p, miR-221-3p, miR-21, miR-125a-3p, LncRNA-MALAT1, LncRNA-NEAT1, miR-145-5p, miRNA-21*). Therefore, many molecules and signaling axes are implicated in the regulation of senescence by the MSC secretome, some of which have controversial effects depending on the cell type analyzed. In addition, RNAs, especially, show an essential role in the modulation of the senescent phenotype and may represent great candidates for rejuvenation approaches. However, further studies on the composition of the MSC-secretome and the mechanism of action of potential candidate molecules are necessary to clarify the role of the secretome in rejuvenation therapies, as well as to assess the safety of these strategies, particularly regarding the risks of malignancy and other adverse effects.

Conclusions

The results revealed the proof-of-principle of the beneficial effects of the MSC-derived secretome from varied sources and in different forms to reverse, at least in part, the senescent phenotype. Thus, the secretome of MSCs could be utilized in rejuvenation strategies to treat senescent cells, including senescent MSCs, allowing extensive expansion of the culture without losing its therapeutic effect. Considering *in vivo* approaches, the secretome could be applied as a therapy for aging-related diseases through the rejuvenation of differentiated cells affected by chronological, biological, or induced aging and even the reversal of stem cell exhaustion.

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Declaration of Competing Interest

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

Author Contributions

Conception and design of the study: KB and AT. Acquisition of the data: KB. Analysis and interpretation of data, drafting or revising the manuscript: KB and AT. Both authors have approved the final article.

Declaration Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT, provided by OpenAI, and Grammarly, provided by Grammarly Inc., to improve the grammar and readability of the manuscript. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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