Received: 13 September 2020 | Revised: 24 October 2020 | Accepted: 27 October 2020

DOI: 10.1002/cbf.3602

REVIEW ARTICLE

CELL BIOCHEMISTRY & FUNCTION WILEY

Ageing and mesenchymal stem cells derived exosomes: Molecular insight and challenges

Mahdi Ahmadi[1] | Jafar Rezaie[2]

[1]Tuberculosis and lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[2]Solid Tumor Research Center, Cellular and Molecular Medicine Research Institute, Urmia University of Medical Sciences, Urmia, Iran

Correspondence
Jafar Rezaie, Solid Tumor Research Center, Cellular and Molecular Medicine Institute, Urmia University of Medical Sciences, Shafa St, Ershad Blvd., P.O. Box: 1138, Urmia 57147, Iran.
Email: rezaie.j@umsu.ac.ir

Ageing induces a great risk factor that participates in progressing various degenerative diseases morbidities. The main characteristic of ageing is the failure in maintaining homeostasis in the organs with a cellular senescence. Senescence is characterized by reduced cell growth, evade cellular death, and acquiring a senescence-associated secretory phenotype (SASP). Mesenchymal stem cells (MSCs) are advantageous cells in regenerative medicine, exerting pleiotropic functions by producing soluble factors, such as exosomes. MSCs and their exosomes (MSCs-Exo) kinetic are affected by ageing and other aged exosomes. Exosomes biogenesis from aged MSCs is accelerated and their exosomal cargoes, such as miRNAs, vary as compared to those of normal cells. Besides, exosomes from aged MSCs loss their regenerative potential and may negatively influence the function of recipient cells. MSCs-Exo can improve ageing and age-related diseases; however, the detailed mechanisms remain yet elusive. Although exosomes-therapy may serve as a new approach to combat ageing, the translation of preclinical results to clinic needs more extensive investigation on exosomes both on their biology and related techniques. Overall, scrutiny on the effect of ageing on MSCs and vice versa is vital for designing novel therapy using MSCs with focus on the management of older individuals.

KEYWORDS
ageing, exosomes, mesenchymal stem cells, senescence

1 | INTRODUCTION

The number of aged population is increasing with a growing load on the healthcare system worldwide. Along with ageing, a substantial raise in the occurrence of age-associated morbidities, such as chronic kidney disease, cardiovascular disease (CVD), neurodegeneration, osteoarthritis (OA), diabetes, osteoporosis, and cancer, are predicted.[1] The degenerative chronic diseases impose a burden on society in case of life quality and financial affairs. It is estimated that the economic costs of health care will be €35 trillion over the next two decades globally.[2] Ageing is a multifarious process that includes cellular mechanisms that senescence plays a pivotal role. During the cellular senescence, cells undergo cell cycle arrest against stress conditions. Senescent cells can also be considered as bystander cells, releasing various bioactive molecules, called the senescence-associated secretory phenotype (SASP). Various cells, including senescent cells, release extracellular vesicles (EVs), such as exosomes, which play important role in inter-cellular communication.[3,4] They transfer biomolecules including RNAs, proteins, and lipids that can participate in physiological functions and the pathogenesis of diseases. Mesenchymal stem cells (MSCs) are multipotent stem cells that participate in regeneration and repair damaged tissues not only via direct differentiation into tissue cells but also through producing different soluble factors, such as exosomes.[5] Exosomes from MSCs (MSCs-Exo) play therapeutic roles in improving different disease models.[6,7] Considering the interplay between exosomes and ageing, therefore, a vital necessity to better understand the mechanisms involved in ageing, especially on MSCs as beneficial cells and how MSCs-Exo can affect ageing for developing novel therapeutic approaches.

2 | EXTRACELLULAR VESICLES

EVs encompass a highly developed system of cell-to-cell communication, by which different kinds of lipids, proteins, and nucleic acids are exchanged between cells.[8] EVs contribute to regulating both physiological and pathological functions of the cells. These vesicles are produced by different cells and are present in almost bio-fluids, so they can affect the function and fate of target cells.[8,9] EVs can target cells through endocytosis, membrane infusion, and ligand-receptor mediated interactions.[10] Classically, EVs can be classified into exosomes, the smallest EVs that are generated from the multi-vesicular body (MVB); and micro-vesicles that are produced from cells through budding of the plasma membrane. Thirdly, the largest EVs, known as apoptotic bodies, are formed by blebbing of apoptotic cells.[8,10] Exosomes are 30 to 150 nm EVs that represent spheroid and cup-shaped morphology using cryoEM technique transmission electron microscopy, respectively.[11] Late endosomes or MVBs are the sources of intraluminal vesicles (ILVs). The ILVs are secreted into the extracellular environment in consequence of exocytosis following the fusion of MVBs with the plasma membrane. Different molecules participate in loading, generation, trafficking, and secretion of exosomes. As mentioned, exosomes contain different types of biomolecules on their surface and lumen; however, they have specific surface proteins, such as tetraspanins (CD81, CD9, and CD63) that make them distinct from other EVs.[8,10] Although exosome isolation and characterization have been further explained, as many articles stay to be published regarding EVs as a whole, the International Society of Extracellular Vesicles (ISEV) provides guiding principles for standardized purification methods, characterization, and isolation of particular EVs.[12] Regarding ISEV guidelines, isolation and characterization of exosomes could be repeated, leading to further reproducible information through different experiments.

3 | AGEING

Ageing is a general term of losing of physiological function over time, both at the cellular and organismal levels, that is regulated by distinct signalling pathways, which lead to physiological dysfunction and malfunction of organs, followed by infirmity and increased probability of death.[13] The underlying mechanisms are thought to be evolutionarily conserved[14,15] and some factors, including epigenetic alterations, telomere shortening, genomic instability, mitochondrial dysfunction, deregulated nutrient sensing machinery, loss of proteostasis, stem cell failure, senescence, and transformed cellular communication,[16] which features result in dysregulated physiological function with ageing,[17-19] amassing of cell and organ harm, failure in renovation capacity, and risk in carcinogenesis.[20-22] Individual organs, tissues, cells, organelles, and biomolecules may possibly age by different rates.[2] Thus, it seems that not all individuals age at the same rate and way.[23]

Long-lasting cell cycle arrest and resistance to cell death are the main properties of cellular senescence. Different stimuli can induce senescence.[24] For example, DNA damage caused by chemical or physical stress stimuli can induce senescence. In this regard, reactive oxygen species (ROS) production induces oxidative stress that damages DNA, protein, and mitochondria, resulting in decreasing homeostasis and inducing senescence.[25] During senescence, nuclei is compacted and enlarged with a morphology associated with stress fibres, reduced adherent property, and accumulation of macromolecules, as well as cellular vacuolization. In addition, ROS promote acidic senescence-associated b-galactosidase (SAbGal) activity, a molecule used as a biomarker of senescence. However, it was suggested that senescence-associated lysosomal a-Lfucosidase (SA-a-Fuc) is a sensitive biomarker.[25] In senescent cells, senescence-associated heterochromatin foci (SAHF) and other factors, including p16INK4a, p27, p21, p53, and pRB, were increased.[26] The SASP factors are chemokines (monocyte chemoattractant protein-1 [MCP-1], IL-8), cytokines (IL-1b and IL-6), growth factors (VEGF, TGFb, HGF, bFGF, and IGF-1), and matrix metalloprotease-1 (MMP-1), −3, −13.[27,28] Despite developments, it is necessary to recognize the mechanisms involved in ageing during the life cycle and not just in the second half of life.

4 | AGEING MANAGEMENT STRATEGIES

Different strategies have been introduced to reduce ageing process and age-related morbidities, including improving lifestyle factors, exercise, and nutrition, improving health and life span.[29] In some cases, the organs transplantation may be lifesaving for patients with age-associated organ damage.[30] For example, in the past 25 years, only in the United States, over two million life-years were added to patients.[31] Furthermore, in the last decade, stem cell-based therapies were introduced as efficient way to regenerate and repair tissue, however, some challenges remain to be resolve for clinical translation. Some concerning such as cell expansion, safety, the suitable cell sources, cancer risk, delivery to the target tissue, proper differentiation, and cost.[32] Consequently, cell-free therapy may be a proper alternative against stem cell therapy. Previous studies have shown that stem cell-derived exosomes have the therapeutic potential regarding regenerative medicine and age-related diseases.[33] Exosomes-therapy (especially MSCs-Exo) exerts beneficial outcomes in regenerative medicine[33,34] (Figure 1). Alterations in cell-to-cell communication may mediate ageing.[16] As mentioned, exosomes are the key mediators of inter-cellular communication; however, their roles in ageing remain largely unknown. Besides, exosomes participate in both physiological and pathological ageing and can be used as a biomarker.[35,36] It seems that investigations on exosomes kinetics may open helpful avenue to uncover mechanisms involved in ageing processes and associated adverse effects on life span reducing ageing process and mortality.

5 | EXOSOMES FROM AGED MSCs

A growing body of study has shown that aged and senescence-induced cells abundantly produce EVs.[4,37] Similarly, the production of exosomes from MSCs increases by late passage cultures or aged

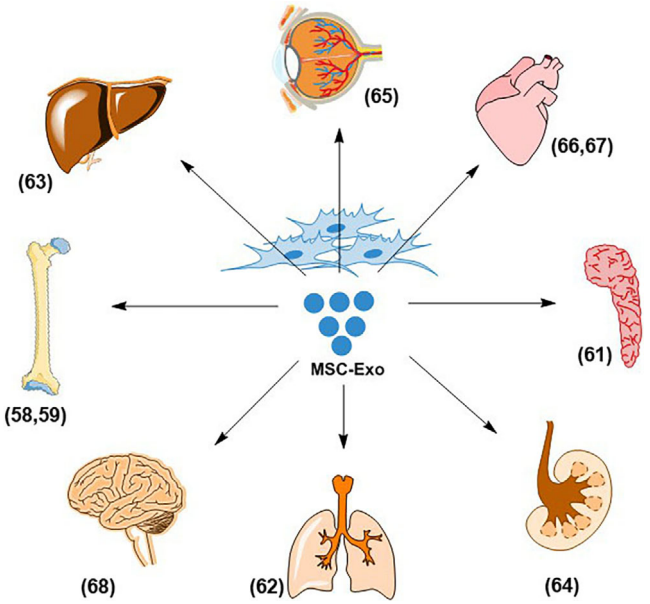

FIGURE 1 The therapeutic potential of mesenchymal stem cell-derived exosomes (MSCs-Exo). Ageing harms functional physiology of key organs. MSCs-Exo can serve as a novel cell-free therapeutic intervention to recover/promote regeneration, improving the human health span. The relevant references are defined below each organ

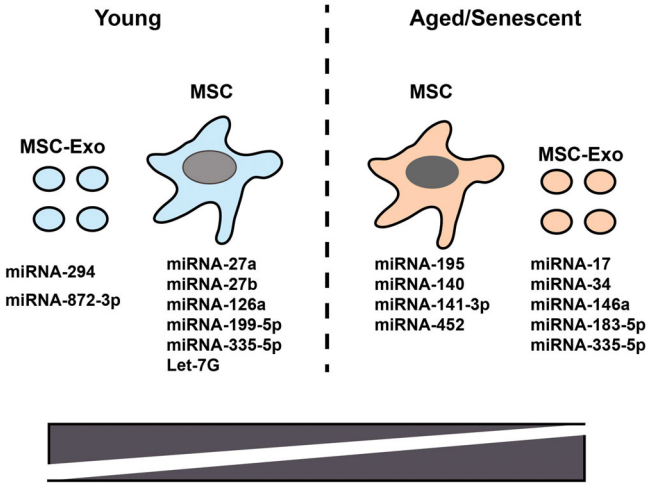

FIGURE 2 Characterizes of miRNAs profile of young and senescent mesenchymal stem cells (MSCs) and their exosomes (MSCs-Exo). With ageing, MSCs abundantly release exosomes with altered cargoes, such as miRNAs. Different miRNAs are modulated in MSCs and MSCs-Exo with ageing/senescence

donors.[38,39] It seems that p53 /TSAP6 axis regulates the production of exosomes from senescent cells. P53, a transcriptional regulator, regulates expression of Rab5B and Rab27B genes, which facilities the endosomal system and exosomes/MVBs trafficking.[40] In addition, ionizing radiation-induced DNA damage can induce p53 protein activation, which promotes TSAP6 activity, in turn, TSAP6 induces exosome generation.[41] The increased secretion rate of exosomes from senescent cells may be a way to remove unwanted, toxic, and mis-folded molecules to maintain cells safe.[42] For example, transporting fragmented DNA by exosomes from cells may inhibit the aberrant activation of DNA damage response pathways and support cellular homeostasis. Secretion of exosomes by senescent cells may be a compensatory response to modulate surrounding environment and enable adjacent cells to respond extra efficiently and rapidly to stress. However, these exosomes may contribute to SASP via transferring pro-senescent signals. Exosomal cargoes of senescent cells differ from the normal ones.[43] Exosomes have been shown to play pivotal roles in reducing inflammation during ageing that is related to the pathogenesis of age-related diseases. Exosomal miRNAs, which are involved in inflammation, may modulate the age-related processes including DNA damage, mitochondrial dysfunction, and senescence.[44] The inflammatory exosomal miRNAs, such as miRNA-20a, miRNA-19b, miRNA-21, miRNA-146a, miRNA-126, and miR-155, have been identified in various cell types. Expression pattern of different miRNAs in MSCs-Exo is modified during age.[38] For instance, expression of miRNA-294 and miRNA-872-3p decreased in MSCs-Exo from old as compared with young rats.[45] Lei et al showed that expression of exosomal miRNA-335-5p and miRNA-146a were decreased and increased, respectively, in late passage MSCs as compared to early passage MSCs, however, expression of both miRNAs up-regulated in aged MSCs.[39] In addition, exosomes from aged bone marrow-derived MSCs of mice contain miRNA-183-5p, which can promote senescence in young MSCs.[46] Several exosomal miRNAs whose expression differs between aged and young MSCs are prepared in Figure 2.[47-50] It is reasonable that exosomes released by senescent and/or aged MSCs show different biological function vs exosomes from young ones.[24] Young MSCs release exosomes that rejuvenate old haematopoietic stem cells and renovate their functions by delivering a high level of lineage commitment and autophagy-related mRNAs.[49] In this regard, the authors concluded that the MSCs-Exo could participate in niche associated ageing of haematopoietic stem cells. Exosome isolated from bone marrow MSCs of aged mice diminished the sensitivity of myocytes, adipocytes, and hepatocytes to insulin in vitro and induced insulin-resistance in vivo.[51] Huang et al declared that young MSCs-Exo improved liposaccharide-induced acute lung injury, whereas aged MSCs-Exo did not display protecting effects.[52] Indeed, these exosomes induced an anti-inflammatory phenotype switch in macrophages. Although the results regarding the roles of exosomes derived from senescent MSCs in the ageing of the organism is limited, however, the idea that the exosomes from senescent MSCs might effect and modulate tissue homeostasis and stem cell niches have been reported.[24] Thus, additional experiments are essential to investigate the role of senescent MSCs-Exo in cell and tissue homeostasis.

6 | EFFECT OF AGED EVs ON MSCs

Besides tissue microenvironment, the SASP in bio-fluids can influence the functions and characteristics of various cells like stem cells,

participating in tissue homeostasis occurring with ageing. Davis et al showed that exosomes derived from the bio-fluid collected young or aged mice represented diverse miRNA profiles and a profound rise in the miRNA-183 in aged exosomes.[46] Incubation of young MSCs with aged exosomes inhibited differentiation ability of MSCs into osteoblasts. They also showed that over-expression of miRNA-183-5p in young MSCs inhibited cell growth and osteogenesis and induced senescence in those cells. These features have been confirmed by Weilner et al who found that exosomes obtained from the blood plasma of elderly donors reduced the osteogenic capacity of young MSCs.[53]

Exosomes/EVs from older women contain abundantly C24:1 ceramide, a sphingolipid involved in cell apoptosis and senescence.[54] MSCs easily can uptake C24:1 ceramide-loaded exosomes, which induce senescence. Exosomes released from Muscle cells of aged mice are enriched with miRNA-34a as compared to young mice. miRNA-34a is associated with inflammation and ageing.[55] Exosomes from myoblasts over-expressing miRNA-34a could decrease the proliferation of MSCs and induced senescence by promoting SA-bGal activity. By in vivo experiments, the authors showed that these exosomes homed to the bone and increased senescence in MSCs ex vivo. They indicated that aged myoblasts may secret senescence-associated exosomes, influencing stem cells in tissues. These data confirm that circulating aged exosomes may induce senescence in MSCs.

7 | DISTINCT EFFECT OF MSCs-Exo ON AGEING

As mentioned above, cellular senescence, the phenotypic changes and ageing, causes a risk factor those results in the progression of different degenerative diseases. The regenerative potential of MSCs-Exo has been reported in various age-related degenerative diseases.[56,57] MSCs-Exo exhibited regenerative properties on bone and cartilage degradation in OA[58,59] and in diabetes,[60] in pancreatic diseases,[61] in lung and liver injury,[62,63] in retinal degenerative diseases,[64] in renal failure,[65] in CVDs,[66] or stroke.[67] The effects of exosomes on the ageing process have been studied in vivo. For example, Zhang et al revealed that biogenesis of exosomes from the hypothalamic neural stem (NSS) was decreased during ageing.[68] In keeping, they showed that treatment with exosomes from healthy cells controlled whole body's ageing via miRNAs. Yoshida et al found that the levels of eNAMPT were decreased in the circulating system with age, however, over-expression of eNAMPT in adipose tissue or administration of eNAMPT-containing exosomes could increase the lifetime of aged mice.[69] Exosomes delivered eNAMPT into recipient cells, increasing NADC synthesis, a factor involved in the ageing process. Nevertheless, the detailed mechanisms of MSCs-Exo elicited effects on age-associated senescence have not been elucidated. A growing body of study showed that proliferative, pluripotency, and metabolic profiles of MSCs are varied by the donor age.[70] Besides, there is evidence that immune-related cargoes of exosomes from MSCs have age-dependent variances and they could be altered using miRNA.[38] A study by Wang and co-workers showed that secretome from human fetal MSCs improves senescence in adult MSCs via decreasing SA-bGal expression and activity in cells, which, in turn, promoted proliferation and osteogenic differentiation of MSCs.[71] Similarly, the supernatant from MSCs modulated senescence in IL1b-treated OA chondrocytes by regulating SA-bGal activity, which, in turn, decreased accumulation of gH2AX foci and actin stress fibres.[72] Furthermore, supernatant collected from MSCs up-regulated sirtuin-1 gene and reduced the oxidative stress and p21 gene expression. In another study, it was reported that MSCs-Exo from normal individuals inhibited SA-bGal activity and gH2AX foci in IL1b-treated osteoblasts obtained from OA individuals.[73] MSCs-EXo have been shown to suppress the production of proinflammatory factors like PGE2 and IL6 and also the oxidative stress. MSCs release exosomes, increasing growth and inhibiting senescent in MSCs promoted to genomic or replicative senescence.[74] Recently, it was demonstrated that incubation of old MSCs with exosomes from young MSCs reduced ageing markers and the mTOR pathway proteins, whereas up-regulated the pluripotency markers in those cells. Further scrutiny showed that exosomal miRNA-188-3p was involved in ageing by regulating the mTOR complex.[75] These findings provide the idea that MSCs-Exo may be used for the progress of novel therapies against ageing-associated diseases.

8 | CHALLENGES IN EXOSOME-THERAPY

Exosome-therapy, cell-free therapy, face some challenges (Figure 3). The International Society for Extracellular Vesicles (ISEV, www.isev.org) is a worldwide society of leading EVs, exosomes, and MVs researchers. ISEV's mission is to develop EVs research globally; however, our

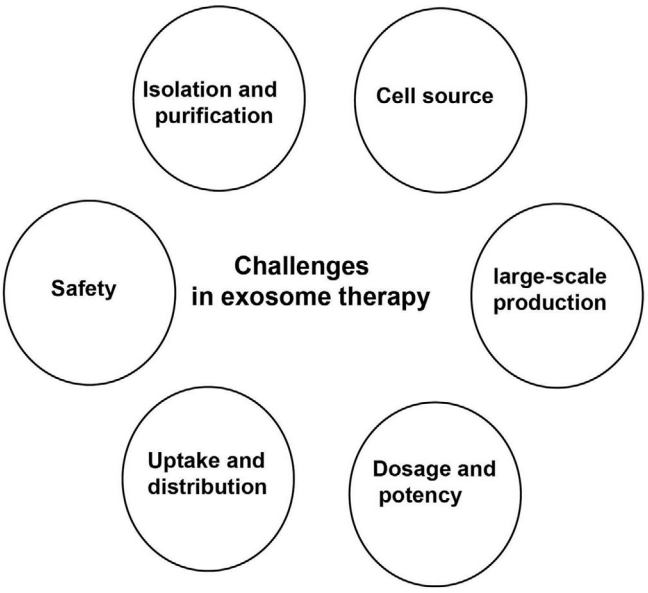

FIGURE 3 Challenges are associated with the clinical application of exosomes in the regeneration of age-affected organs. MSCs-Exo means mesenchymal stem cell-derived exosomes

TABLE 1 Clinical trials on MSCs-Exo recorded by 9/6/2020

Source cell	Identifier number	Condition	Phase	Status
Allogeneic adipose mesenchymal stem cells	NCT04313647	Lung Injury	I	Recruiting
Umbilical cord-derived mesenchymal stem cell	NCT04213248	Dry Eye	I and II	Recruiting
Allogeneic adipose mesenchymal stem cells	NCT04276987	Coronavirus	I	Not yet recruiting
Umbilical cord-derived mesenchymal stem cell	NCT04356300	Multiple Organ Failure	Not applicable	Not yet recruiting
Allogeneic mesenchymal stem cell	NCT03384433	Cerebrovascular Disorders	I and II	Completed
Mesenchymal stem cell	NCT03437759	Macular Holes	I	Recruiting
Allogeneic Mesenchymal stem cells	NCT04173650	Epidermolysis Bullosa	I and II	Not yet recruiting

knowledge about exosomes is increasing and further validation is essential. For example, proper exosome production, purification, and targeted delivery are concerns regarding regenerative medicine (Figure 3). Further studies, which take exosomes cargoes, as well as exosomes safety into account, will need to be undertaken.[76] Exosomes isolation and purification need an optimized method to harvest large amounts of exosomes for clinical applications. Among methods for exosome isolation, ultracentrifugation techniques is still a standard and relatively cheap method, however, it suffers from biological contamination and exosome loss because of the heterogeneity of exosomes.[77] In addition, the production of exosomes in a large-scale is essential for clinical application. Conventional cell culture is not affordable in terms of cost and time. 2D culture techniques are generally used to obtain exosomes, but they are labour-intensive and produce a low level of exosomes. Using 3D culturing techniques, or bioreactors, increases efficiency and exosomes yield,[78,79] however, these approaches require more media and more frequent passage. On the other hand, although these larger-scale methods maximize cell culture surface area for expansion, heterogeneity in cells and consequently in exosomes remain a challenge.

Another challenge is the determination of dosage and potency of exosomes for clinical application because exosomes are heterogeneous across different progenitor cells and different animal models used for exosomes therapy, as well as there are differences in cellular characteristics and assessment.[80] Consequently, extrapolating exosomes dosage from preclinical models to clinical trials also needs precise consideration. Since exosomes from various cells represent different characteristics, standards could be more efficiently optimized when a tissue is selected and methods used to deliver exosomes to target tissue. In addition, the distribution and uptake of exosomes and exosomes half-life should be identified for avoiding off-target tissue accumulation and producing optional exosomes to reduce non-target of exosomes.[81] Despite therapeutic features of exosomes, especially those derived from MSCs, Food and Drug Administration (FDA) has not approved translating exosomes to clinical trials due to the heterogeneity in exosomes, cell sources, and isolation, as well as complication in validation techniques. Thus, extensive investigation on exosome stability in bio-fluids, safety, potency, and quality should be done and conserved throughout shipping and processing conditions. However, there are some companies manufacturing exosome-based products and therapies that use exosomes. Among companies, Aegle Therapeutics Company has received Investigational New Drug (IND) application from the FDA. Overall, although exosomes are likely alternative to cell-based therapies, therapeutic hurdles and challenges remain when translating to clinical studies.

9 | CLINICAL TRIALS

Consistent with preclinical studies on the application of exosomes in regenerative medicine, application of MSCs-Exo has been recently considered in clinical trials. Clinical use of MSCs-Exo is currently limited. This is partly because the translation of MSCs-based therapies from the animal models to the clinic needs resolving of critical factors. There are currently seven clinical trials evaluating MSCs-Exo reported to Clinicaltrials.gov (Table 1).

10 | CONCLUSION

Ageing may influence MSCs and their exosomes both in generation rate and type of cargo. With increasing age, MSCs and exosomes loss their capability for proliferation, wound healing, differentiation, and angiogenesis. Increased production of a SASP component, such as exosomes, contributes to cellular senescence and ageing in recipient cells. Preclinical studies have established the beneficial properties of MSCs-Exo in improving age-related diseases. Translation of preclinical outcomes into clinic faces different challenges associated with exosomes kinetics and biology. Success in exosomes-therapy may depend on the physiological function of aged/senescent MSCs because these cells may loss therapeutic function and reveres/reduce the efficacy of the treatment. A proper understanding of the detailed mechanisms involved in the senescence process may help us to develop therapies to improve tissue regeneration and manage the loss of organs function and diseases associated with ageing.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

ORCID

Jafar Rezaie https://orcid.org/0000-0001-8175-3730

REFERENCES

1. Suzman R, Beard JR, Boerma T, Chatterji S. Health in an ageing world—what do we know? *Lancet*. 2015;385:484-486.
2. G Shiels P, Ritzau-Reid K. Biological ageing, inflammation and nutrition: how might they impact on systemic sclerosis? *Curr Aging Sci*. 2015;8:123-130.
3. Hassanpour M, Rezaie J, Nouri M, Panahi Y. The role of extracellular vesicles in COVID-19 virus infection. *Infect Genet Evol*. 2020;85:104422.
4. Takasugi M, Okada R, Takahashi A, Chen DV, Watanabe S, Hara E. Small extracellular vesicles secreted from senescent cells promote cancer cell proliferation through EphA2. *Nat Commun*. 2017;8:1-11.
5. Lai RC, Yeo RWY, Lim SK. Mesenchymal stem cell exosomes. *Semin Cell Dev Biol*. 2015;40:82-88. https://doi.org/10.1016/j.semcdb.2015.03.001.
6. Harrell CR, Jovicic N, Djonov V, Volarevic V. Therapeutic use of mesenchymal stem cell-derived exosomes: from basic science to clinics. *Pharmaceutics*. 2020;12:474.
7. Khaksar M, Sayyari M, Rezaie J, Pouyafar A, Montazersaheb S, Rahbarghazi R. High glucose condition limited the angiogenic/cardiogenic capacity of murine cardiac progenitor cells in in vitro and in vivo milieu. *Cell Biochem Funct*. 2018;36:346-356.
8. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol*. 2014;30:255-289.
9. Jabbari N, Akbariazar E, Feqhhi M, Rahbarghazi R, Rezaie J. Breast cancer-derived exosomes: tumor progression and therapeutic agents. *J Cell Physiol*. 2020;235:6345-6356.
10. Van Niel G, d'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol*. 2018;19:213-228.
11. Pol E, Coumans F, Grootemaat A, et al. Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. *J Thromb Haemost*. 2014;12:1182-1192.
12. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. 2018;7:1535750.
13. Kirkwood TB, Austad SN. Why do we age? *Nature*. 2000;408:233-238.
14. Xu Y, Sun Z. Molecular basis of Klotho: from gene to function in aging. *Endocr Rev*. 2015;36:174-193.
15. Kenyon CJ. The genetics of ageing. *Nature*. 2010;464:504-512.
16. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;153:1194-1217.
17. Baker DJ, Wijshake T, Tchkonia T, et al. Clearance of $p16^{Ink4a}$-positive senescent cells delays ageing-associated disorders. *Nature*. 2011;479:232-236.
18. Baker DJ, Childs BG, Durik M, et al. Naturally occurring $p16^{Ink4a}$-positive cells shorten healthy lifespan. *Nature*. 2016;530:184-189.
19. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell*. 2005;120:513-522.
20. Campisi J. Aging, cellular senescence, and cancer. *Annu Rev Physiol*. 2013;75:685-705.
21. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature*. 2001;414:105-111.
22. Tukmechi A, Rezaee J, Nejati V, Sheikhzadeh N. Effect of acute and chronic toxicity of paraquat on immune system and growth performance in rainbow trout, *Oncorhynchus mykiss*. *Aquacult Res*. 2014;45:1737-1743.
23. Fulop T, Larbi A, Witkowski JM, et al. Aging, frailty and age-related diseases. *Biogerontology*. 2010;11:547-563.
24. Lunyak VV, Amaro-Ortiz A, Gaur M. Mesenchymal stem cells secretory responses: senescence messaging secretome and immunomodulation perspective. *Front Genet*. 2017;8:220.
25. Li Y, Wu Q, Wang Y, Li L, Bu H, Bao J. Senescence of mesenchymal stem cells. *Int J Mol Med*. 2017;39:775-782.
26. Larsson L-G. Oncogene-and tumor suppressor gene-mediated suppression of cellular senescence. *Semin Cancer Biol*. 2011;21(6):367-376.
27. Herranz N, Gil J. Mechanisms and functions of cellular senescence. *J Clin Invest*. 2018;128:1238-1246.
28. Rodier F. Detection of the senescence-associated secretory phenotype (SASP). *Cell Senescence*. Totowa, NJ: Humana Press; 2013:165-173.
29. Shiels PG, McGlynn LM, MacIntyre A, et al. Accelerated telomere attrition is associated with relative household income, diet and inflammation in the pSoBid cohort. *PLoS One*. 2011;*6*:e22521.
30. Giwa S, Lewis JK, Alvarez L, et al. The promise of organ and tissue preservation to transform medicine. *Nat Biotechnol*. 2017;35:530-542.
31. Rana A, Gruessner A, Agopian VG, et al. Survival benefit of solid-organ transplant in the United States. *JAMA Surg*. 2015;150:252-259.
32. Steinbeck JA, Studer L. Moving stem cells to the clinic: potential and limitations for brain repair. *Neuron*. 2015;86:187-206.
33. Panagiotou N, Davies RW, Selman C, Shiels PG. Microvesicles as vehicles for tissue regeneration: changing of the guards. *Curr Pathobiol Rep*. 2016;4:181-187.
34. Keshtkar S, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. *Stem Cell Res Ther*. 2018;9:63. https://doi.org/10.1186/s13287-018-0791-7.
35. Shiels PG, Stenvinkel P, Kooman JP, McGuinness D. Circulating markers of ageing and allostatic load: a slow train coming. *Pract Lab Med*. 2017;7:49-54.
36. D'anca M, Fenoglio C, Serpente M, et al. Exosome determinants of physiological aging and age related neurodegenerative diseases. *Front Aging Neurosci*. 2019;11:232.
37. Beer L, Zimmermann M, Mitterbauer A, et al. Analysis of the secretome of apoptotic peripheral blood mononuclear cells: impact of released proteins and exosomes for tissue regeneration. *Sci Rep*. 2015;5:16662.
38. Fafián-Labora J, Lesende-Rodriguez I, Fernández-Pernas P, et al. Effect of age on pro-inflammatory miRNAs contained in mesenchymal stem cell-derived extracellular vesicles. *Sci Rep*. 2017;7:1-12.
39. Lei Q, Liu T, Gao F, et al. Microvesicles as potential biomarkers for the identification of senescence in human mesenchymal stem cells. *Theranostics*. 2017;7:2673-2689.
40. Fujii M, Kawai Y, Endoh M, Hossain MN, Nakabayashi K, Ayusawa D. Expression of RAB27B is up-regulated in senescent human cells. *Mech Ageing Dev*. 2006;127:639-642.
41. Lespagnol A, Duflaut D, Beekman C, et al. Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in TSAP6/Steap3-null mice. *Cell Death Differ*. 2008;15:1723-1733.
42. Takahashi A, Okada R, Nagao K, et al. Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. *Nat Commun*. 2017;8:15287.
43. Urbanelli L, Buratta S, Sagini K, Tancini B, Emiliani C. Extracellular vesicles as new players in cellular senescence. *Int J Mol Sci*. 2016;17:1408.
44. Prattichizzo F, Giuliani A, Sabbatinelli J, et al. Extracellular vesicles circulating in young organisms promote healthy longevity. *J Extracell Vesicles*. 2019;8:1656044.
45. Wang Y, Fu B, Sun X, et al. Differentially expressed microRNAs in bone marrow mesenchymal stem cell-derived microvesicles in young and older rats and their effect on tumor growth factor-β1-mediated epithelial-mesenchymal transition in HK2 cells. *Stem Cell Res Ther*. 2015;6:185.
46. Davis C, Dukes A, Drewry M, et al. MicroRNA-183-5p increases with age in bone-derived extracellular vesicles, suppresses bone marrow

stromal (stem) cell proliferation, and induces stem cell senescence. *Tissue Eng Part A*. 2017;23:1231-1240.
47. Ganguly P, El-Jawhari JJ, Giannoudis PV, Burska AN, Ponchel F, Jones EA. Age-related changes in bone marrow mesenchymal stromal cells: a potential impact on osteoporosis and osteoarthritis development. *Cell Transplant*. 2017;26:1520-1529.
48. Okada M, Kim HW, Matsu-ura K, Wang YG, Xu M, Ashraf M. Abrogation of age-induced MicroRNA-195 rejuvenates the senescent mesenchymal stem cells by reactivating telomerase. *Stem Cells*. 2016;34:148-159.
49. Kulkarni R, Bajaj M, Ghode S, Jalnapurkar S, Limaye L, Kale VP. Intercellular transfer of microvesicles from young mesenchymal stromal cells rejuvenates aged murine hematopoietic stem cells. *Stem Cells*. 2018;36:420-433.
50. Vono R, Jover Garcia E, Spinetti G, Madeddu P. Oxidative stress in mesenchymal stem cell senescence: regulation by coding and noncoding RNAs. *Antioxid Redox Signal*. 2018;29:864-879.
51. Su T, Xiao Y, Xiao Y, et al. Bone marrow mesenchymal stem cells-derived exosomal MiR-29b-3p regulates aging-associated insulin resistance. *ACS Nano*. 2019;13:2450-2462.
52. Huang R, Qin C, Wang J, et al. Differential effects of extracellular vesicles from aging and young mesenchymal stem cells in acute lung injury. *Aging (Albany NY)*. 2019;11:7996-8014.
53. Weilner S, Schraml E, Wieser M, et al. Secreted microvesicular miR-31 inhibits osteogenic differentiation of mesenchymal stem cells. *Aging Cell*. 2016;15:744-754.
54. Khayrullin A, Krishnan P, Martinez-Nater L, et al. Very long-chain C24:1 ceramide is increased in serum extracellular vesicles with aging and can induce senescence in bone-derived mesenchymal stem cells. *Cells*. 2019;8:37.
55. Fulzele S, Mendhe B, Khayrullin A, et al. Muscle-derived miR-34a increases with age in circulating extracellular vesicles and induces senescence of bone marrow stem cells. *Aging (Albany NY)*. 2019;11:1791-1803.
56. Phinney DG, Pittenger MF. Concise review: MSC-derived exosomes for cell-free therapy. *Stem Cells*. 2017;35:851-858.
57. Chen L, Qu J, Xiang C. The multi-functional roles of menstrual blood-derived stem cells in regenerative medicine. *Stem Cell Res Ther*. 2019;10:1-10.
58. Cosenza S, Ruiz M, Toupet K, Jorgensen C, Noël D. Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis. *Sci Rep*. 2017;7:16214. https://doi.org/10.1038/s41598-017-15376-8.
59. D'Arrigo D, Roffi A, Cucchiarini M, Moretti M, Candrian C, Filardo G. Secretome and extracellular vesicles as new biological therapies for knee osteoarthritis: a systematic review. *J Clin Med*. 2019;8:1867.
60. Fan B, Li C, Szalad A, et al. Mesenchymal stromal cell-derived exosomes ameliorate peripheral neuropathy in a mouse model of diabetes. *Diabetologia*. 2020;63:431-443.
61. Ahmed SM, Morsi M, Ghoneim NI, Abdel-Daim MM, El-Badri N. Mesenchymal stromal cell therapy for pancreatitis: a systematic review. *Oxid Med Cell Longev*. 2018;2018:1-14.
62. Lee C, Mitsialis SA, Aslam M, et al. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. *Circulation*. 2012;126:2601-2611.
63. Wang X, Wang S, Zhou Y, et al. BM-MSCs protect against liver ischemia/reperfusion injury via HO-1 mediated autophagy. *Mol Med Rep*. 2018;18:2253-2262.
64. Ding SLS, Kumar S, Mok PL. Cellular reparative mechanisms of mesenchymal stem cells for retinal diseases. *Int J Mol Sci*. 2017;18:1406.
65. Missoum A. Recent updates on mesenchymal stem cell based therapy for acute renal failure. *Curr Urol*. 2019;13:189-199.
66. Suzuki E, Fujita D, Takahashi M, Oba S, Nishimatsu H. Therapeutic effects of mesenchymal stem cell-derived exosomes in cardiovascular disease. *Adv Exp Med Biol*. 2017;998:179-185.
67. Doeppner TR, Herz J, Görgens A, et al. Extracellular vesicles improve post-stroke neuroregeneration and prevent postischemic immunosuppression. *Stem Cells Transl Med*. 2015;4:1131-1143.
68. Zhang Y, Kim MS, Jia B, et al. Hypothalamic stem cells control ageing speed partly through exosomal miRNAs. *Nature*. 2017;548:52-57.
69. Yoshida M, Satoh A, Lin JB, et al. Extracellular vesicle-contained eNAMPT delays aging and extends lifespan in mice. *Cell Metab*. 2019;30:329-342.e5.
70. Fafián-Labora J, Fernández-Pernas P, Fuentes I, et al. Influence of age on rat bone-marrow mesenchymal stem cells potential. *Sci Rep*. 2015;5:16765.
71. Wang B, Lee WY-W, Huang B, et al. Secretome of human fetal mesenchymal stem cell ameliorates replicative senescence. *Stem Cells Dev*. 2016;25:1755-1766.
72. Platas J, Guillén MI, del Caz MDP, et al. Paracrine effects of human adipose-derived mesenchymal stem cells in inflammatory stress-induced senescence features of osteoarthritic chondrocytes. *Aging (Albany NY)*. 2016;8:1703-1717.
73. Tofiño-Vian M, Guillén MI, Pérez del Caz MD, Castejón MA, Alcaraz MJ. Extracellular vesicles from adipose-derived mesenchymal stem cells downregulate senescence features in osteoarthritic osteoblasts. *Oxid Med Cell Longev*. 2017;2017:1-12.
74. Liu S, Mahairaki V, Bai H, et al. Highly purified human extracellular vesicles produced by stem cells alleviate aging cellular phenotypes of senescent human cells. *Stem Cells*. 2019;37:779-790.
75. Fafián-Labora J, Morente-López M, Sánchez-Dopico MJ, et al. Influence of mesenchymal stem cell-derived extracellular vesicles in vitro and their role in ageing. *Stem Cell Res Ther*. 2020;11:1-12.
76. Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. *Acta Pharm Sin B*. 2016;6:287-296.
77. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. *Theranostics*. 2017;7:789-804.
78. Phan J, Kumar P, Hao D, Gao K, Farmer D, Wang A. Engineering mesenchymal stem cells to improve their exosome efficacy and yield for cell-free therapy. *J Extracell Vesicles*. 2018;7:1522236.
79. Watson DC, Bayik D, Srivatsan A, et al. Efficient production and enhanced tumor delivery of engineered extracellular vesicles. *Biomaterials*. 2016;105:195-205.
80. Willis GR, Kourembanas S, Mitsialis SA. Toward exosome-based therapeutics: isolation, heterogeneity, and fit-for-purpose potency. *Front. Cardiovasc. Med.* 2017;4:63.
81. Morishita M, Takahashi Y, Nishikawa M, Takakura Y. Pharmacokinetics of exosomes—an important factor for elucidating the biological roles of exosomes and for the development of exosome-based therapeutics. *J Pharm Sci*. 2017;106:2265-2269.

How to cite this article: Ahmadi M, Rezaie J. Ageing and mesenchymal stem cells derived exosomes: Molecular insight and challenges. *Cell Biochem Funct*. 2020;1–7. https://doi.org/10.1002/cbf.3602