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Extracellular vesicles from mesenchymal stromal cells: therapeutic perspectives for senescence targeting in osteoarthritis

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Abstract (150)

Osteoarthritis (OA) is a common age-related disease that correlates with a high number of senescent cells in joint tissues. Senescence has been reported to be one of the main drivers of OA pathogenesis, in particular via the release of senescence-associated secretory phenotype (SASP) factors. SASP factors are secreted as single molecules and/or packaged within extracellular vesicles (EVs), thereby contributing to senescent phenotype dissemination. Targeting senescent cells using senolytics or senomorphics has therefore been tested and improvement of OA-associated features has been reported in murine models. Mesenchymal stromal cells (MSCs) and their derived EVs (MSC-EVs) are promising treatments for OA, exerting pleiotropic functions by producing a variety of factors. However, functions of MSCs and MSC-EVs are affected by aging. In this review, we discuss on the impact of the senescent environment on functions of aged MSC-EVs and on the anti-aging properties of MSC-EVs in the context of OA.

Keywords: mesenchymal stromal cell, extracellular vesicle, senescence, aging, osteoarthritis, regenerative medicine

1. Introduction

Osteoarthritis (OA) is the most common joint disorder characterized by cartilage deterioration, calcification in the form of osteophytes and subchondral sclerosis and, low-grade synovitis. Several risk factors have been identified including genetics, obesity, repeated traumas, metabolic disorders, but the most prevalent one is age. This degenerative and debilitating disease affects over half of individuals aged 65 years and older, which yields around 30 million adults in the US population [1]. It is estimated that the number of people affected will double in the coming years to reach nearly 67 million by 2030 [2]. Senescence is one of the hallmarks of aging that has been implicated in the pathogenesis and progression of most of aging-associated diseases, including OA (for review, see [3]). However, the exact mechanism connecting senescence and OA pathology remains unclear. Senescent cells are cell cycle-arrested and release a secretome containing various bioactive molecules, called the senescence-associated secretory phenotype (SASP). These molecules can be released as single molecules or within extracellular vesicles (EVs) that contribute to the pathogenesis of OA and the degradation of joint tissues. Targeting senescent cells therefore represents a novel approach for treating OA [4].

Among the therapeutic strategies being investigated for the treatment of OA is the use of mesenchymal stromal/stem cells (MSCs). They have proved efficacy in non-clinical models of OA by decreasing inflammation and protecting cartilage and bone from degradation and, in the clinics by improving pain and functional parameters ([5, 6], for meta-analysis and review [7]). More recently, MSC-derived EVs (MSC-EVs) have been reported to recapitulate most of the beneficial effects of parental MSCs. They improved the phenotype of OA chondrocytes in vitro [8] and exerted a therapeutic effect in murine models of OA [9, 10] or

in cartilage repair [11]. EV-based therapies may therefore represent novel alternatives to cells to combat aging via the secretion of regenerative factors and the depletion of senescent cells. Besides, EVs from aged or senescent MSCs may lose their regenerative potential and negatively influence the function of recipient cells through the release of SASP factors. In this review, we discuss the therapeutic role of MSC-EVs in OA and focus on senescence-associated features of OA.

2. Mesenchymal stromal cells and extracellular vesicles

EVs are mediators in the crosstalk between cells and contribute to the regulation of tissue homeostasis and pathological processes. These vesicles are produced by virtually all cell types and are detected in all body fluids. They are classified into three major subtypes depending on their size and biogenesis: exosomes (from 40 to 150 nm diameter) from endosomal origin, microvesicles (or microparticles, ectosomes; 100 nm to 1 µm) budding from the plasma membrane and apoptotic bodies (larger than 500 nm) evolving from cell disruption during apoptosis [12]. Nonetheless, currently used isolation methods achieve only enrichment in two main categories of heterogeneous populations of EVs: small size EVs (less than 150 nm) and medium size EVs (120 to 600 nm) from other soluble molecules contained in culture medium or fluids [13, 14]. There is no consensus marker to identify EV subtypes but presence of a lipid bilayer as well as size and surface (CD9, CD63, CD81, flotillin) and cytosolic (HSP70, TSG101, ALIX,..) markers are widely used to characterize EVs. The composition of EVs coming from different cell compartments and distinct intracellular regulatory processes is variable and their cargo differs. Likewise, EV cargoes mostly reflect the source and state of parental cells and obviously, their function thereof. This is the reason

why MSC-EVs are largely investigated as novel cell-free therapeutic weapons in a large variety of diseases [15].

Although MSC-EVs have proved therapeutic potential in different preclinical settings, their mechanism of action is still poorly understood. Different omics approaches have identified bioactive molecules (proteins, lipids, miRNAs) playing various roles in biological processes relevant for tissue repair, such as inflammation, angiogenesis, extracellular matrix remodelling, apoptosis, proliferation [15]. There are consensual data reporting that EVs isolated from different sources of MSCs have beneficial effects but few have compared the efficacy of different sources of MSC-EVs in parallel within a single study. Nevertheless, in OA, induced pluripotent stem (iPS) cells-derived MSC-EVs were shown to be more effective than synovium-derived MSC-EVs to alleviate OA histological score [10]. In this last paper, the mechanism underlying efficacy was not investigated but the effect of reprogramming on cell features, notably rejuvenation and inhibition of senescence might be one possible explanation.

3. Common features of senescence and osteoarthritis

3.1. Cellular senescence features

Senescence is defined as irreversible growth arrest and resistance to apoptosis in response to intrinsic signals (aging, replicative stress) and extrinsic signals, including DNA damage, oncogenic signalling and oxidative stress [16]. It is characterized as a complex process involving dysregulation of various cellular functions and molecular pathways. Hallmarks of senescence are cell-cycle arrest, expression of cyclin-dependent kinase

inhibitors (CDKI, namely p16INK4A, p21, p27, p53, pRB), hypertrophic morphological changes, production of acidic senescence-associated β-galactosidase (SA-β-Gal), accumulation of DNA damage and senescence-associated heterochromatin foci (SAHF), metabolic dysfunction and, production of SASP factors, including interleukin 1 (IL1), IL6, IL8, monocyte chemoattractant protein 1 (MCP-1), matrix metalloproteinases (MMP1, MMP3), vascular growth factor (VEGF), hepatocyte growth factor (HGF), insulin growth factor 1 (IGF1), basic fibroblast growth factor (bFGF) (Fig. 1). Senescence has a primary beneficial role of tumour suppressor by up-regulating CDKIs in response to oncogenic signals but is also important for regulating tissue repair or embryogenesis [17]. Nevertheless, its contribution in aging-related diseases may be seen as a negative impact by boosting inflammation, matrix degradation and over-growth. Indeed, secretion of the SASP factors contributes to disease onset and progression in many, if not all, aging-related diseases, such as OA.

3.2. Senescence and osteoarthritis associated features

The close relationship between OA and senescence has been reviewed elsewhere [3]. In OA patients, the number of senescent cells in cartilage, synovial membrane, infrapatellar fat pad and subchondral bone correlates with age. Senescent p16INK4A^{+} chondrocytes, synovial fibroblasts, osteoblasts and bone marrow (BM) progenitors are increased in OA [18-20]. Their accumulation is one of the key process contributing to disease progression by the secretion of SASP factors generating an inflammatory and degradative tissue environment similar to that is found in OA [21]. In the mouse model of collagenase-induced osteoarthritis, the transient accumulation of p16INK4A^{+} senescent cells is observed in the synovial tissue, at

day 24 after OA induction [22]. Senescence is also a characteristic of post-traumatic OA and age-related OA [23, 24].

Joint cells in OA exhibit common hallmarks of senescence, including telomere attrition and increased expression of p53, the CDKIs p16INK4A and p21, enhanced generation of reactive oxygen species (ROS) and increased SAHF and SASP (for review, see [25]) (Fig. 1). The SASP comprises pro-inflammatory cytokines, such as IL1β, IL6, metalloproteinases, such as MMP13 and a desintegrin and metalloproteinase with thrombospondin motifs-5 (ADAMTS-5) and growth factors, including VEGF, that contribute to inflammation, matrix degradation and osteophyte formation, all key features of OA. These circulating factors participate to the deregulation of tissue homeostasis in aged mice. Using the heterochronic parabiosis model, it was clearly demonstrated that young mice from the young/aged parabiosis group experienced higher proteoglycan loss and lower chondrocyte proliferation in cartilage four months after surgery than the young/young group [26]. The RUNX2/SOX9 ratio was also increased by a 5-fold factor in the cartilage samples from young/aged group indicative of hypertrophy induction and initiation/progression of OA. By contrast, aged mice from the aged/young parabiosis group displayed higher chondrocyte proliferation, lower osteophyte formation and a normalized balance between anabolic and catabolic markers [27]. Interestingly, injection of recombinant growth differentiation factor-11 (GDF11) reproduced similar improvement on cartilage matrix synthesis in aged mice. Novel results showed that EVs isolated from plasma of young mice increase the longevity of aged mice by 10% [28]. This effect was attributed to their content in high amounts of extracellular nicotinamide phosphoribosyltransferase (eNAMPT) and the central role of NAD^+-SIRT1 axis in the aging process. Data therefore suggest that EVs are central in the secretome of senescent

cells and may contribute to premature senescence by lacking anti-aging factors and spreading pro-aging factors, thereby contributing to cartilage degeneration [29].

4. Impact of age on the functions of mesenchymal stromal cells-derived EVs

EV secretion by MSCs was shown to increase with their senescence status and age while the quantity of proteins decreased [30]. Aged MSCs display altered functions and support pro-aging signals that are found within released EVs (Fig. 2). Growth rate, cell migration as well as differentiation potential are impacted with age or in an aged environment and the miRNA profile in their EVs is altered [31]. Indeed, circulating EVs from aged human donors were found to negatively impact the osteogenic differentiation potential of primary adipose tissue-derived MSCs (ASCs) in a donor age-dependent manner [32]. This decreased osteogenic capacity of aged MSC-EVs was correlated with lower amount of galectin-3. Similarly, EVs isolated from the BM of aged mice were shown to inhibit the osteoblastogenic capacity of young MSCs. This inhibition was mediated by miR-183-5p, which reduced cell proliferation and differentiation while it increased senescence and oxidative stress [33]. More recently, EVs isolated from osteoblasts from patients with OA or osteoporosis exerted a negative effect on MSCs, as shown by increased apoptotic rate and decreased osteogenic potential [34]. Effect of aging on the immunosuppressive properties of MSCs has not been reported. However, some miRNAs associated with the immune profile of MSCs were shown to be decreased in EVs from aged MSCs and proposed to be related to the loss of their immunotherapeutic function [35]. Another study reported that aged MSC-EVs were not able to alleviate inflammation in LPS-induced lung injury model [36]. They did not reduce the recruitment of macrophages and were incapable of promoting M2 macrophage polarization.

The pro-angiogenic activity and pro-wound healing effect of oxidative stress-induced senescent MSC-EVs are also decreased compared to healthy MSCs [37]. The downregulation of miR-146a contributed to the impaired pro-angiogenic capacity of senescent MSC-EVs. Evidence is therefore accumulating to confirm the loss of function of MSCs and their EVs with aging.

Finally, SASP factors contained within EVs from aged individuals can induce premature senescence in MSCs. An increased number of muscle-derived miR-34a-5p-containing EVs was detected in the serum of aged mice and these EVs were described to negatively impact MSC viability and increase their senescence [38]. Accordingly, blood-derived EVs carrying C24:1ceramide were found in higher numbers in older women and shown to contribute to the induction of senescence in MSCs [39]. Finally, addition of aged MSC-EVs on young MSCs down-regulated the pluripotency markers Nanog, Oct4 and up-regulated the senescence markers Vinculin, LMNA and mTOR while young MSC-EVs had an opposite effect on aged MSCs [30]. Although not directly reported, it is likely that MSCs, in constant or repeated contact with aged EVs, will produce senescent EVs with altered cargo and dysregulated functions. Besides altered functions, EVs from aged or senescent MSCs could induce senescence in neighbouring cells through the SASP. Still, data reporting the pro-senescent effect of MSC-EVs are sparse. An interesting study has nicely demonstrated that aged MSC-EVs transfer negative regulators of autophagy causing aging of hematopoietic stem cells (HSCs) [40]. Aged MSCs possess activated protein kinase B (AKT) signalling, which reduces the amount of several autophagy-related mRNAs transferred in their EVs while increasing the levels of miR-37 and miR-34a, two miRNAs targeting autophagy-related mRNAs. Likewise, incubation of aged MSC-EVs on young MSCs increased the levels of aging markers and decreased the levels of multipotency markers [30]. However, as indicated above, these

examples are unique and further evidence is needed to firmly conclude that senescence-induced MSC-EVs transfer pro-aging or pro-senescent signals. No data is available on the role of senescent MSC-EVs on OA chondrocytes and synoviocytes, in vitro or in vivo, that could indicate that pro-senescent signals could not be counterbalanced by reparative or protective signals carried by such EVs, even though such functions are partially impaired in aged MSCs.

5. Rejuvenation of aged MSCs and improvement of EV function

Deterioration of the functionality of MSCs with aging has been largely demonstrated as discussed above. Restoring the properties of aged MSCs to that of young MSCs has therefore received much attention in the field of regenerative medicine. Together with decreased expression of anti-aging genes, including TERT, SIRT1, BCL2 and increased SA-β-Gal staining, the expression of miR-140, miR-146a/b, miR-195 is enhanced in aged MSCs [41]. Silencing miR-195 in aged MSCs was shown to restore the expression of anti-aging mediators, comprising TERT, SIRT1, FOXO1, phosphorylated AKT and to improve their therapeutic effect in a model of cardiac infarction. Another study has shown that rapamycin or p70S6K knock-down ameliorated chemical- or oxidative stress-induced senescence in MSCs [42]. Strategies that target oxidative stress in MSCs are promising for counteracting the detrimental effect of age and senescence in MSCs (for review see [43]). Novel data indicated that infant MSC-EVs can rejuvenate elderly MSCs by inhibiting the accumulation of ROS, reducing SA-β-Gal positive cell number, inducing proliferation and upregulating the expression of cytokines that promoted wound healing in diabetic mice [44]. A similar study recently reported the interest of umbilical cord (UC)-derived MSC-EVs to rejuvenate senescing BM-MSCs, thereby exhibiting alleviated aging phenotype as well as increased self-renewal capacity and

telomere length [45]. This improvement was at least partly mediated by the transfer of proliferating cell nuclear antigen (PCNA) into aging BM-MSCs. Rejuvenated MSCs exhibited increased bone formation potential after subcutaneous implantation on bone graft substitutes, higher wound repair capacity and ameliorated aging-related angiogenic dysfunction. Aged MSCs were also rescued using a treatment with LY294002, an inhibitor of AKT signalling, and their derived EVs transferred higher amounts of autophagy marker-related mRNAs, such as BECLIN-1, ATG7, LC3a, LC3b, SIRT1-3 and lower levels of miR-17 and miR-34a, resulting in increased therapeutic activity of MSCs and improved rejuvenating capacity on aged HSCs [40]. Induced pluripotent stem cells (iPSCs) were reported to produce 16-fold more EVs than MSCs that were able to rescue both exhausted passaged MSCs as a premature model of replicative senescence and progerin-expressing MSCs as a genetically-induced senescence model [46]. Both iPSC-EVs and young MSC-EVs were efficient to improve the senescent state and function of aged MSCs. The mechanism of action of iPSC-EVs was through the delivery of peroxidoxin antioxidant enzymes that reduced intracellular ROS levels. Interestingly, embryonic stem cells (ESC)-EVs can ameliorate the senescent phenotype of MSCs both *in vitro* and *in vivo* and delay bone loss in aged mice [47]. These results indicate that EVs can rejuvenate tissue-resident stem cells and thereby prevent age-related disorders. It is noteworthy that the expansion conditions of MSCs can exert positive effects on senescence-associated changes since 3D culture was shown to reduce telomere attrition and SA-β-Gal expression and, improve mitochondrial function and differentiation potential of MSCs [48]. Therefore, a better understanding of culture conditions and factors that could reduce senescence-associated features would increase the safety and effectiveness of MSCs in large scale production process. In that respect, culture conditions or priming strategies to generate enhanced MSCs that could combine anti-senescence and

improved reparative functions should be developed in the future for generating clinical grade EVs with superior capacities.

6. Therapeutic effect of MSC-EVs in osteoarthritis

6.1. Chondroprotective effect of MSC-EVs on OA characteristics in joint tissues

The beneficial action of MSC-EVs on OA chondrocyte phenotype was demonstrated *in vitro* by the up-regulation of the anabolic markers (type II collagen and aggrecan) together with the inhibition of catabolic (MMP-13, ADAMTS5), inflammatory (induced nitric oxide synthase (iNOS)) markers as well as apoptosis [9]. A similar study reported the decrease of inflammatory NFκB signalling, COX2 expression, collagenase activity and increased production of glycosaminoglycans (GAG), aggrecan, type II collagen, SOX9 expression in OA chondrocytes [49]. By contrast, lack of benefit on MMP activity and PGE2 secretion was shown using ASC-EVs [50]. Likewise, production of anabolic markers was reduced by synovial-derived MSC-EVs but this effect was reversed by over-expressing miR-140-5p in MSCs before EV isolation [51]. Nevertheless, two recent systematic reviews of literature reported a general tendency towards anti-inflammatory, anti-apoptotic, pro-migratory, pro-proliferative, anti-catabolic and pro-autophagic effect of MSC-EVs whatever the source [52, 53]. Since, this pleiotropic effect of MSC-EVs on OA human chondrocytes was also shown using human MSC-EVs and reported to be mediated through the down-regulation of IL1β-mediated activation of ERK1/2, PI3K/AKT, p38, TAK1, and NF-κB signaling pathways [54]. Interestingly, another study identified lncRNA H19 contained in EVs as involved in the mechanism of action of MSC-EVs [55]. LncRNA H19 acts as endogenous sponge of miR-29b-

3p, which targets FOXO3, thereby promoting migration, ECM secretion and suppressing apoptosis and senescence in chondrocytes. In addition, the anti-inflammatory and anti-catabolic properties of MSC-EVs were recently confirmed on chondrocytes and further demonstrated on OA synoviocytes indicating that all joint tissues might benefit from MSC-EV therapy [56].

Several studies have reported the therapeutic effect of MSC-EVs *in vivo* using distinct models of OA [51, 57, 58]. Of interest, some studies evaluated different strategies to enhance MSC-EV-based treatment in OA. The importance of miR-31 in this process was recently demonstrated [59]. Human synovial MSC-EVs containing high amount of miR-31 were reported to alleviate cartilage damage and inflammation in knee joints in a model of OA induced by transection of the medial collateral ligament and the medial meniscus. Over-expression of miR-31 in MSC-EVs further improved cartilage preservation. Mechanistically, miR-31 targets KDM2A, which inhibits the transcription of E2F1, resulting in enrichment of E2F1 and increased chondrocyte proliferation and migration. Hypoxia pre-treatment of MSCs before EV isolation was also described to improve OA symptoms in the destabilization of medial meniscus (DMM) rat model, as visualized by reduced osteophyte formation and OA histological score as well as preservation of cartilage extracellular matrix proteins [60]. Hypoxia was shown to enhance the amount of miR-216a-3p in EVs resulting in its effective transfer in target chondrocytes and down-regulation of JAK2. JAK2/STAT3 signalling pathway inhibition promoted proliferation, migration and inhibited apoptosis of chondrocytes. Repeated injections of MSC-EVs are another mean to enhance or at least prolong treatment efficacy. Although not compared with a single injection, repeated injections of MSC-EVs over the disease course resulted in significant cartilage protection in two models of OA in rats (monosodium iodoacetate (MIA) and DMM) with high reduction in the number of $IL1\beta^{+}$

chondrocytes and $CD86^+$ synoviocytes [61]. These recent data highlight the possibility of generating MSC-EVs with enhanced therapeutic properties and pave the way for clinical translation.

6.2. Senotherapeutic effect of MSC-EVs on senescent features of joint tissues

A senotherapeutic effect of MSC-EVs has been demonstrated in some cases. EVs recovered from early passage MSCs were shown to alleviate the aging phenotype of senescent MSCs undergoing replicative senescence (>passage 10), as shown by reduction of SA-β-Gal staining, p21 and p53 expression, IL1α and IL6 secretion and, γ-H2AX production [46]. Human decidua-derived MSC-EVs not only promoted the proliferation, migration and differentiation of dermal fibroblasts but also improved their senescent state [62]. In another model, aged HSCs, which have been rejuvenated following a brief exposure to young MSC-EVs, were able to engraft at higher levels after bone marrow transplantation and this effect was attributed to the up-regulation of autophagy markers in EVs [40]. Consistently, aged MSCs treated with an inhibitor of AKT signalling produced EVs that were more efficient to rescue aged HSCs. In addition, the rejuvenating power of inhibitor-treated MSC-EVs was even higher than that of young EVs on HSC function in primary and secondary BM transplantation experiments. The modulation of miRNA levels in MSC-EVs was reported to improve aging-related features. Indeed, down-regulation of miR-29b-3p in MSC-EVs significantly ameliorated insulin resistance in aged mice via SIRT1 regulation [63].

Together with the demonstration that MSC-EVs directly impact on the senescent phenotype of target cells, a number of reports have shown beneficial effects on aging-

related diseases and in OA (for review see [64]). However, only few data exist on a direct effect of MSC-EVs on OA-associated senescence. The first report on the effect of MSC secretome on the senescent phenotype of OA chondrocytes used the conditioned medium of MSCs [65]. The authors described a therapeutic benefit as indicated by decreased SA-β-Gal activity, γH2AX foci number, actin stress fiber formation as well as p21 and p16 mRNA expression. Our unpublished results using human ASC-EVs clearly indicated their role in preventing senescence features of OA chondrocytes, including SA-β-Gal activity, stress fiber formation and γH2AX foci, using two models of etoposide- and IL1β-induced senescence. MSC-EVs have also been found to reduce senescent features in OA osteoblasts (SA-β-Gal expression, γ-H2AX production) while reducing inflammatory (IL6 and PGE2) and oxidative stress (HNE) markers [66]. The data are nonetheless scarce and more studies are required to confirm the anti-senescence capacity of MSC-EVs and for further understanding the underlying mechanism of action. This would allow to increase the therapeutic properties of MSC-EVs as discussed below.

7. Discussion

Senescence is now seen as a hallmark of OA, and even more as a driver in OA pathogenesis, suggesting that treatments targeting senescence might be relevant. And indeed, intra-articular injections of senolytics and senomorphics can eliminate senescent cells in cartilage and synovium tissues and, improve OA features [67, 68]. The interest of MSCs and their EVs as senomorphics has been pointed out in the present review. However, a number of crucial parameters, in particular MSC source, parental cell density, passage number, culture conditions, MSC priming or method of EV isolation may influence the yield and functions of MSC-EV preparations as discussed in a recent systematic review of the literature on MSC-EVs-based treatments in pre-clinical settings [69]. Besides

the development of standards and consensus on processes for EV isolation that will help improving overall efficacy of EV therapeutics, the age or the senescence state of parental MSCs are rarely discussed. How this can impact on MSC-EV functions needs to be further investigated and this issue is particularly important for age-related degenerative diseases such as OA when autologous MSCs are used.

Aged BM-MSCs have impaired proliferation, senescence and chondrogenic response whereas ASCs or muscle-derived MSCs do not exhibit such effect [70]. A decline of BM-MSC number with aging has also been suggested but the most relevant change is the reduced capacity of aged cells to differentiate into osteoblasts with increased bias toward adipogenic differentiation (for review, see [71]). BM-MSC-EVs have been shown to positively regulate osteoblastic activity and bone formation in vivo, notably via transfer of miRNAs [72]. A decline in such activity is therefore expected in aged MSC-EVs but the likely most impacting effect of aging is the up-regulation of the SASP and the down-regulation of anti-oxydative and anti-aging properties as previously described. This implies that in an allogenic perspective, MSCs originating from young donors should be favored to reduce the effect of aging on the functionality of EVs.

Likewise, among the different sources of MSCs that can be used for EV preparations, perinatal MSCs might be more functional, notably with respect to their anti-inflammatory functions. Indeed, Wharton's Jelly-derived MSCs exhibit enhanced expression of immunosuppressive factors, particularly leukocyte antigen G6 (HLA-G6), indoleamine-2,3-dioxygenase (IDO), prostaglandin E2 (PGE2) and, suppress mitogen-induced T lymphocyte responses to a greater extent than BM-MSCs or ASCs that were isolated from adult tissues [73, 74]. In OA, a recent meta-analysis reports that BM-MSCs, ASCs and UC-MSCs are all effective in clinical trials, yet ASCs outperform BM-MSCs for relieving pain and UC-MSCs were more effective for improving function [75]. It has to be noticed that this analysis included only one study using UC-MSCs and it cannot be excluded that results were mostly related to inter-individual heterogeneity rather than to tissue source. Accordingly, great variations between selection criteria and expansion processes of MSCs between the clinical trials

targeting OA have been highlighted elsewhere, which suggests that the optimal cell source is still speculative [76]. None reported a possible effect of senescence- or age-related features of used MSCs. Further studies are therefore required to definitely conclude that perinatal tissue-derived MSCs could be a more appropriate tissue source than adult MSCs for isolating EVs.

A possibility to circumvent inter-individual heterogeneity in MSC samples or processes, and therefore their derived EVs, would be to use immortalized clonal MSCs or iPS-derived cells. A potential issue with immortalized MSCs is safety. MSC-EVs are not capable of proliferation and therefore cannot form tumors but they might affect tumor growth. Contradictory results on pro- or anti-tumorigenic effects have been reported to date with naïve MSCs [77-80]. However, a MYC-transformed MSC line does not exhibit tumorigenic activity and its derived EVs do not inhibit or promote tumor growth in vivo [81, 82]. Contrary to parental iPS cells, iPS-EVs or EVs from iPS-derived differentiated cells display the advantages of low immunogenicity, no risk of tumor formation and have been shown to have therapeutic potential in regenerative medicine (for review, see [83]). Interestingly, iPS-MSC-EVs can mitigate aging in a model of aging-related arterial stiffness and hypertension by promoting SIRT1, AMPKa and endogenous NOS (eNOS) expression [47]. Again, to our knowledge, a lack of data exists on the possible role of these immortalized MSCs- or iPS cells-derived EVs on senescence features in OA or other degenerative diseases. For safety reasons, the absence of transgene for immortalized MSC-EVs and of reprogramming factors in iPS-EVs must be validated before clinical applications. However, such approaches should guarantee the unlimited supply of EVs with high inter-batch reproducibility.

Finally, MSC priming before or during the production of EV-containing conditioned medium may be relevant for enhancing the anti-aging or reparative properties of MSCs and treating OA or other degenerative diseases. Hypoxia is a known promoter of MSC chondrogenesis but it can also increase the capacity of MSCs to promote the production of cartilage matrix by chondrocytes and decrease apoptosis [84]. Hypoxia priming of MSCs resulted in the production of EVs with higher capacities to promote M2 polarization of macrophages and this effect was attributed to higher levels of miR-21-5p

that down-regulated PTEN [85]. Nevertheless, intravenous injection of these EVs promoted tumor growth by increasing cancer cell survival in a xenograft model. In a rat model of OA, EVs from MSCs cultured under hypoxia exerted a higher protective effect than MSCs cultured under normoxia, both on cartilage degradation and osteophyte formation [60]. In vitro, hypoxia-primed MSC-EVs enhanced the proliferation, migration and anabolism of chondrocytes while reducing their apoptosis and, this effect was associated to the up-regulation of miR-216a-5p. Priming of ASCs with γ-interferon (IFN-γ) was recently shown to modulate the miRNA cargo of derived EVs [86]. Interestingly, the most highly modulated miRNAs were associated to matrix or immune cell-related pathways as well as "skeletal and musculoskeletal disorders". In addition, TGFβ1-primed MSCs-derived EVs carrying higher amounts of miR-135b were also reported to attenuate cartilage damage in an in vivo model of OA through M2 polarization of macrophages [87]. Therefore, hypoxic priming, IFN-γ- or TGFβ1-priming of MSCs are effective and promising approaches to optimize EVs-mediated treatment of OA. Since priming was associated to the increase of specific miRNAs involved in cartilage-protective functions, production of EVs from genetically modified MSCs may be another option for OA treatment. Such strategy has been effective to increase chondrocyte proliferation and anabolism as well as alleviate cartilage damage in vivo using over-expression of miR-26a-5p, miR-31-5p, miR-92a-3p, miR-140-5p and miR-155-5p in MSCs [51, 59, 88-90]. Although most of these miRNAs may be correlated with senescence modulation, miR-140-5p was shown to play a protective role in OA by retarding chondrocyte senescence [91]. The impact of primed MSCs-derived EVs on senescence markers in target tissues is rarely reported and should be investigated in future studies.

8. Conclusions

Available data indicate that MSC-EVs are powerful tools in suppressing or at least delaying OA features both *in vitro* on chondrocytes, osteoblasts and synoviocytes, the major cell components in the joint and, *in vivo*, in different murine and rat preclinical models of OA.

Increasing evidence indicates that MSC-EVs can protect from cellular senescence induction; it is shown in particular for MSCs isolated from young individuals or from rejuvenated iPSCs. There is an overlap between senescence and OA markers that makes difficult to unravel whether MSC-EVs act through targeting factors involved in inflammation, catabolism, oxidative stress or senescence. Nevertheless, cellular senescence is seen as a primary driver of OA pathogenesis and senotherapeutic strategies have proved efficacy to reduce OA [68]. Future investigation is needed to design effective MSC-EV-based treatments for OA-associated senescence and envision clinical translation.

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Declarations of interest

The authors disclose any financial or personal conflict of interest.

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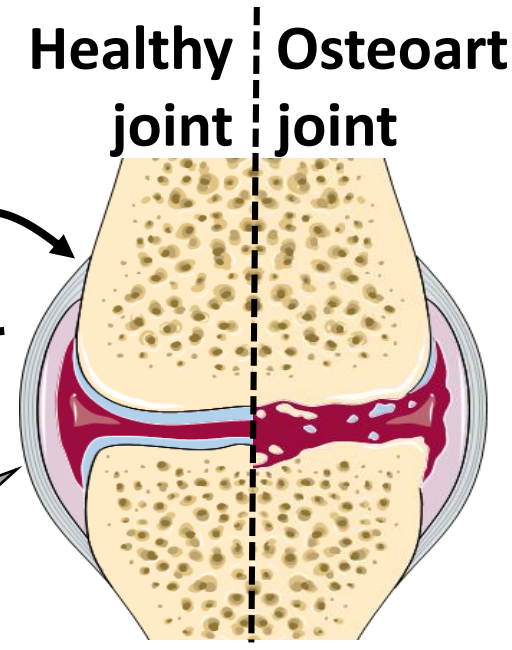
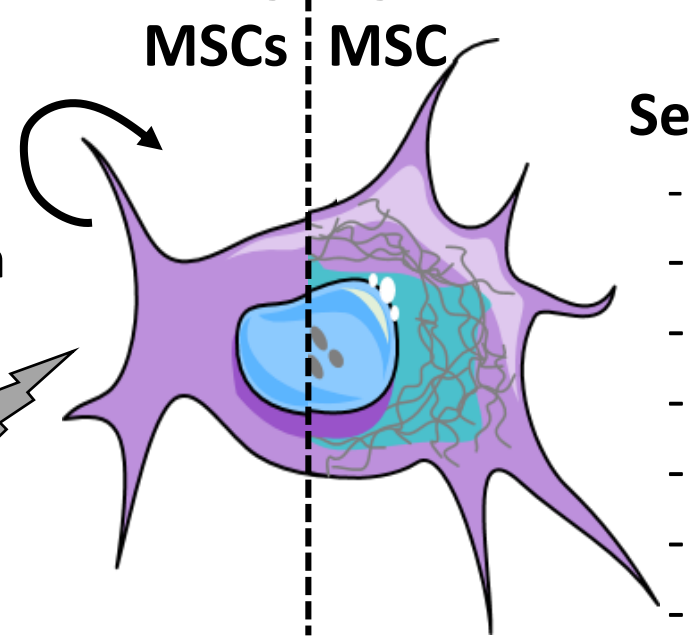
Figure 1



Intrinsic signals
Ageing
Genetics
Metabolic disorder

Extrinsic signals
Trauma

OA features
- Cartilage degradation, bone sclerosis, osteophyte formation, synovial inflammation
- ROS production
- Inflammatory and catabolic factor production (IL1β, IL6, MMP13, ADAMTS5, VEGF)
- Telomere attrition
- SA-β-gal positive cell accumulation
- p53, p16, p21 expression increase

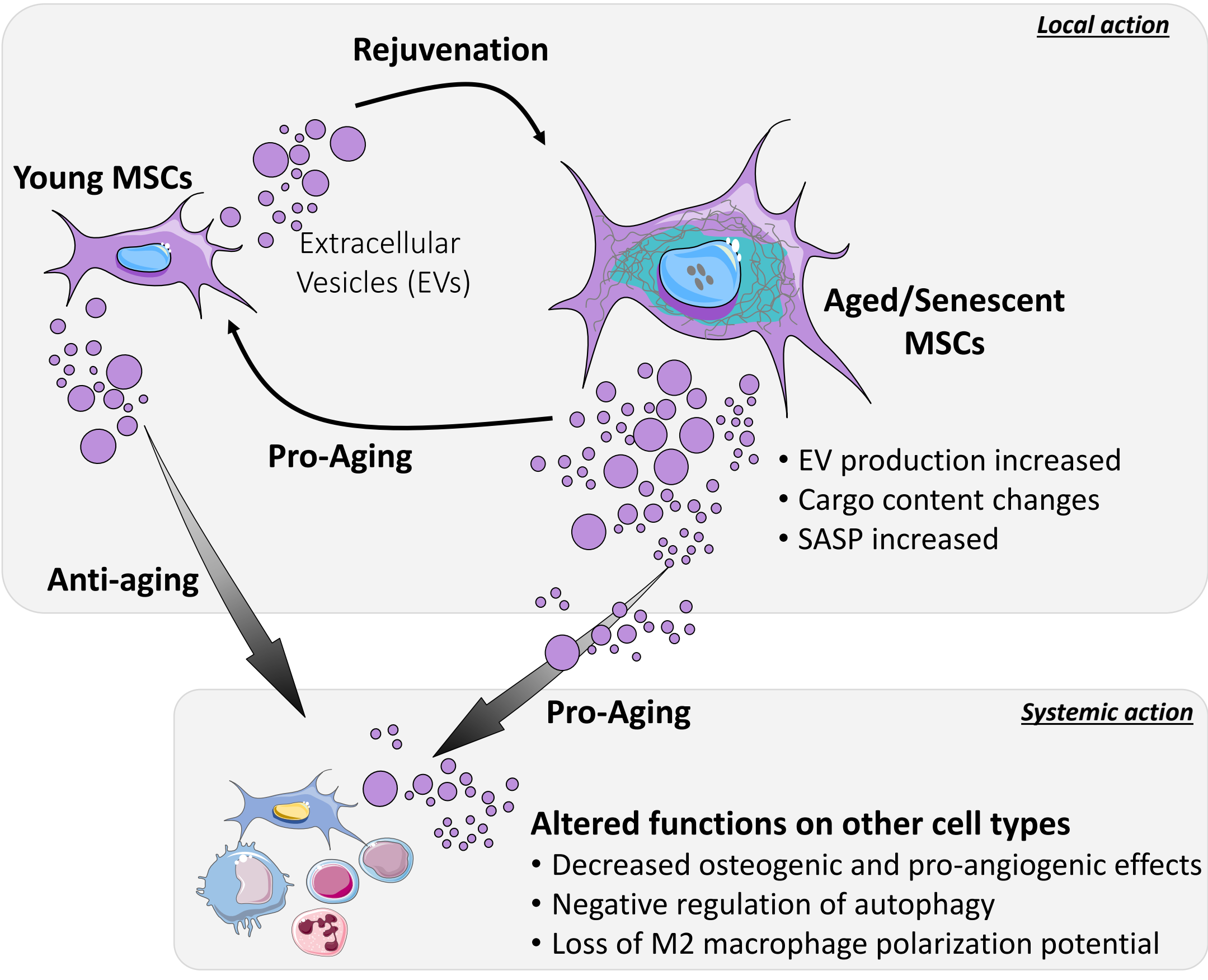


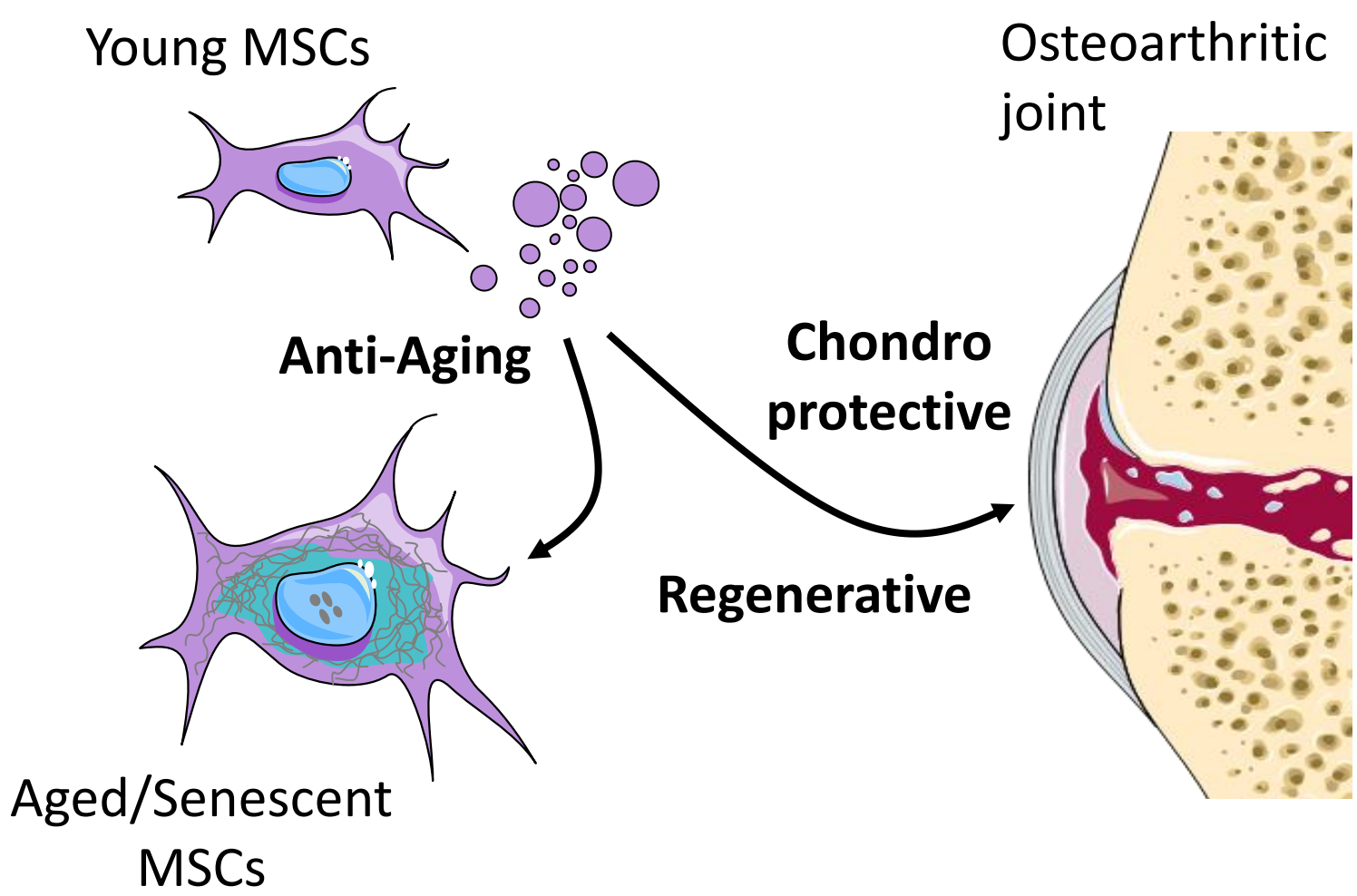
Intrinsic signals
Ageing
Telomere attrition

Extrinsic signals
DNA damage
Oncogenic stress
Oxidative stress

Senescent features
- Cell cycle arrest and expression of CDKI
- DNA damage
- SASP (IL1β, IL6, MMP13, VEGF)
- SAHF
- SA-β-gal activity
- Metabolic dysfunction (ROS production)
- Resistance to apoptosis

Figure 2


Local action
Rejuvenation
Young MSCs
Extracellular Vesicles (EVs)
Aged/Senescent MSCs
Pro-Aging
• EV production increased
• Cargo content changes
• SASP increased
Anti-aging
Pro-Aging
Systemic action
Altered functions on other cell types
• Decreased osteogenic and pro-angiogenic effects
• Negative regulation of autophagy
• Loss of M2 macrophage polarization potential


Young MSCs
Anti-Aging
Aged/Senescent
MSCs
Osteoarthritic
joint
Chondro
protective
Regenerative