Extracellular Vesicles as Communicators of Senescence in Musculoskeletal Aging

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ABSTRACT
Extracellular vesicles (EVs), including exosomes and microvesicles, are released by numerous cell types. EVs are now acknowledged as playing a critical role in cell–cell communication in healthy aging as well as in age-related diseases. Recently it was shown that senescence, a key hallmark of aging, increases the secretion of EVs. Moreover, EVs can transport proteins and microRNAs (miRNAs) that are key components of the senescence-associated secretory phenotype (SASP). Here we review evidence that SASP-related miRNAs are involved in musculoskeletal degeneration with aging. Specifically, senescence-related miRNAs are elevated in EVs released by skeletal muscle myocytes and fibro-adipogenic progenitor cells with aging and disuse atrophy, respectively. Many of these same senescence-related miRNAs are detected in EVs from the synovial fluid of patients with osteoarthritis, and these miRNAs can contribute to cartilage degeneration. Finally, senescence-associated miRNAs are secreted from bone marrow–derived stem (stromal) cells impacting neighboring hematopoietic stem cells and circulating in the blood. The senescence-associated miRNA mir-34a, which is known to target Wnt and Notch pathways as well as the cell survival factors Sirt1 and Bcl2, is detected in EVs from human and animal subjects with muscle atrophy, bone loss, and osteoarthritis. These findings suggest that suppressing the secretion of EV-derived, senescence-related miRNAs, such as miR-34a, or increasing levels of competing endogenous long noncoding RNAs, such as MALAT1 that inhibit miR-34a, may help to improve musculoskeletal function with aging. © 2022 The Authors. JBMR Plus published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research.

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Introduction
Degenerative musculoskeletal diseases, such as osteoporosis, osteoarthritis, and sarcopenia, contribute to significant morbidity and disability in populations around the world. The prevalence of these diseases increases with age, and as the size of the older population increases globally, these diseases place a greater burden on patients, their families, and public health systems.(1) It is now understood that multiple age-related disorders affecting various organs and tissues may have some common underlying causes. Specifically, increased levels of oxidative stress and circulating inflammatory cytokines can negatively impact multiple organs and tissues simultaneously. This fact is underscored by parabiosis studies demonstrating that, on the one hand, circulating factors in aged animals can suppress regenerative processes in young animals and that, on the other hand, young blood can enhance regeneration and cognition in aged animals.(2–4) Numerous circulating factors have been implicated in these systemic changes, such as transforming growth factor beta 1, Notch family members, and age-associated factors like Klotho.(5–8) Recently it has been suggested that the cargo of circulating extracellular vesicles (EVs), including exosomes and microvesicles, is significantly altered with age and with age-related diseases, such as Alzheimer’s disease, and that a more “youthful” EV cargo could reverse some degenerative, age-related changes.(6,7) A key factor driving EV release with aging is senescence. Senescent cells, which have exited the cell cycle and express markers such as p16 and p21, accumulate with age. These cells can promote senescence in neighboring cells through the secretion of factors collectively termed the senescence-associated secretory phenotype (SASP).(9) Notably, a large number of SASP proteins are detected in EVs, and miRNAs associated with senescence are also abundant within EVs (Table 1).(10–12) Furthermore, senescence itself is found to induce EV secretion.(13,14) In fact, senescent cells secrete 20% to 30% more EVs than nonsenescent cells.(15) These findings suggest that EVs may be important communicators of senescence in both a local and systemic manner.
We previously showed that skeletal muscle cells secrete senescence-associated miRNAs in EVs with age\(^{16}\) and disuse atrophy,\(^{17}\) and others have identified similar changes in EVs derived from senescent chondrocytes.\(^{18}\) Here we review the evidence for EVs as communicators of senescence in musculoskeletal tissues and identify potential interventions to target these EVs as an approach for the prevention and treatment of age-related musculoskeletal diseases. Original research papers were found using National Institutes of Health (NIH) PubMed (accessed on June–July 2022), including search terms such as “senescence + osteoarthritis + extracellular vesicles,” “senescence + osteoporosis + extracellular vesicles,” and “senescence + sarcopenia + extracellular vesicles.” Other searches also included “miRNA” as an additional search term. Papers were selected based on relevance and a preliminary abstract screening. A number of the articles were already present in the authors’ libraries and were included where appropriate.

EVs, senescence, and muscle atrophy

Myoblasts and mature myotubes are known to secrete EVs both in vitro and in vivo.\(^{19}\) EV secretion is increased with exercise,\(^{20,21}\) possibly through membrane (sarcolemma) damage that leads to calcium influx and exosome release.\(^{22}\) Exposure of cells to inflammatory cytokines with aging is associated with accumulation of the sphingolipid ceramide and ceramide can, in turn, induce senescence.\(^{23,24}\) Importantly, ceramide accumulation also triggers exosome secretion,\(^{25}\) and exosomes are known to be highly enriched in ceramide.\(^{26}\) Muscle-derived EVs are therefore likely to be secreted in conditions associated with muscle contraction and muscle aging.

We have found that the senescence-associated miRNA miR-34a accumulates with age in mouse skeletal muscle,\(^{16}\) and others have observed the same age-related increase in miR-34a in human skeletal muscle.\(^{27}\) We previously used alpha sarcoglycan (SGCA) as a surface marker to isolate muscle-derived EVs from serum and found that circulating, muscle-derived EVs from older animals are enriched in miR-34a.\(^{16}\) Mir-34a is associated with aging and senescence in other tissues, including the heart\(^{28}\) and brain,\(^{29}\) and is known to target Wnt and Notch pathways, as well as cell survival factors such as Sirt1 and Bcl2.

Interestingly, miR-34a overexpression in muscle cells leads to ceramide accumulation,\(^{20}\) and we have found that miR-34a overexpression in muscle cells leads to their release via EVs.\(^{16}\) Together these findings suggest that factors such as oxidative stress that induce miR-34a expression may in turn trigger the export of miR-34a via EVs by stimulating ceramide accumulation (Fig. 1). Local and circulating EV-derived miR-34a may then communicate senescence to recipient tissues such as bone.\(^{16}\) These findings suggest that muscle-derived EVs may contribute to age-related bone loss via a bystander effect, whereby senescent cells induce senescence in nearby cells and tissues.

Myoblasts and myocytes are not the only cells in skeletal muscle that secrete EVs. Fibro-adipogenic progenitor cells (FAPs) are known to have a substantial effect on muscle satellite cells via their secretome.\(^{31}\) Recently it was shown that small molecule therapy using histone deacetylase inhibitors could “tune” FAPs to release EVs that promote muscle regeneration via their miRNA cargo.\(^{32}\) We utilized a single hindlimb immobilization model to demonstrate that muscle disuse atrophy could induce senescence in FAPs. Specifically, 2 weeks of disuse increases the expression of interleukin-1 beta (IL-1\(\beta\)), a key SASP factor,\(^{33}\) as well as its receptor IL-1R\(^{33}\) IL-1\(\beta\) co-localizes with the senescence marker p16 in FAPs in this immobilization model.\(^{33}\) We have used PDGFR\(\alpha\) as a surface marker to isolate FAP-derived EVs from whole skeletal muscle.\(^{17}\) MiRNA profiling of these FAP-derived EVs reveals that a number of senescence-associated miRNAs, including let-7 family members and miR-181a, are increased in muscle with disuse immobilization (Fig. 1). These miRNAs target mitochondrial membrane solute carriers, such as SLC25A3, which decreases in muscle with immobilization\(^{17}\) and in muscle biopsies from sarcopenic patients with hip fracture.\(^{34}\) Future studies might be directed at identifying EVs positive for either SGCA or PDGFR\(\alpha\) in circulation and in bone marrow interstitial fluid to determine their role in age-related tissue dysfunction.

Osteoarthritis and senescence-associated EVs

EVs are known to circulate in synovial fluid and are released by chondrocytes and synoviocytes.\(^{35,36}\) We\(^{37}\) and others\(^{38}\) have...
found that a number of senescence-associated miRNAs, such as miR-34a, mirR-155, and miR-181a, are identified in synovial fluid from patients with OA (Fig. 2). Importantly, there are noticeable sex differences in the microRNA profile of EVs derived from the synovial fluid of OA patients (Fig. 2), with males expressing more typical senescence-associated miRNAs than females. Our work revealed elevated levels of miR-34a in samples from male OA patients, whereas other studies found increased miR-34a in plasma, cartilage, and synovium of both male and female OA patients. Importantly, intra-articular injection of miR-34a alone can induce an OA phenotype in rodents, consistent with other studies showing that EV-derived miR-34a can communicate senescence in chondrocytes, whereas senolytic therapy attenuates this effect. These findings suggest that EVs are key communicators of senescence in the limb joint microenvironment and may contribute to the development and progression of degenerative joint disease. This conclusion is further supported by data showing that EVs enriched in Connexin 43 released from senescent chondrocytes “spread” senescence to neighboring cells.

EVs as communicators of senescence in aging bone microenvironment

The balance of bone formation and bone resorption is mediated by crosstalk among a variety of cell types in the bone marrow microenvironment, including bone marrow stromal (stem) cells (BMSCs), osteocytes, osteoblasts, osteoclasts, hematopoietic stem cells, and bone marrow adipocytes. These various cell types have all been found to secrete EVs that can impact neighboring cells. For example, the muscle-derived factor myostatin can stimulate the release of osteocyte-derived EVs carrying the miRNA miR-218, which in turn suppresses bone formation by osteoblasts. Increases in senescence-associated miRNAs with age in EVs are associated with a loss of bone marrow stem cells and impaired bone formation. BMSCs are known to secrete EVs enriched in miR-31, which can stimulate bone resorption by osteoclasts, whereas osteoclast-derived EVs carrying miR-214 can suppress bone formation by osteoblasts. Several studies have found miR-34a to increase with age in EVs secreted by BMSCs, and miR-34a suppresses osteoblast differentiation as well as bone formation by differentiated osteoblasts.

Total circulating levels of miR-34a (not solely EV-derived miR-34a) are inversely correlated with bone mineral density in older adults, and circulating miR-34a is increased with ovariectomy and positively correlated with bone loss in rodents. Furthermore, EVs from aged bone matrix transfer microRNAs such as miR-483-5p and miR-2861, which stimulate vascular calcification and bone marrow adipogenesis. These data support the role of senescence-associated miRNAs and their transport by EVs as important pathways for age-related bone loss. Of note, EVs from senescent BMSCs can also impact muscle satellite cells, underscoring the importance of bone-muscle interactions. Suppressing the uptake of these BMSC-derived EVs may have the potential to prevent sarcopenic changes in muscle.

Discussion and Conclusions

The field of geroscience has contributed a number of new and exciting discoveries to our understanding of musculoskeletal aging. Perhaps foremost among these is the recognition that cellular senescence increases with age in multiple tissues, including musculoskeletal tissues, contributing to organ dysfunction with aging. A key mechanism by which senescence impacts multiple organs and tissues is via the SASP, so that senescence is communicated to other cells and tissues in a paracrine manner.

Fig. 2. Venn diagram showing senescence-associated microRNAs detected in EVs from the synovial fluid of male and female osteoarthritis (OA) patients. Males tend to express more senescence-associated miRNAs than females, indicating that the cellular changes that occur between males and females with aging and joint degeneration are likely to differ.
Although EVs are known to play an important role in cell–cell communication, their function as mediators of the SASP has only recently been acknowledged. This is due in part to multiple studies indicating that senescent cells enhance their secretion of EVs relative to nonsenescent, healthy cells. A broad examination of EV cargo catalogued in databases such as Vesiclepedia demonstrates that SASP factors, such as inflammatory cytokines and senescence-associated miRNAs termed “GeroMirs,” are abundant in EVs. These data suggest that EVs are an essential component of the SASP and, thus, are likely to be important in age-related musculoskeletal diseases. These SASP-associated miRNAs detected in EVs may ultimately serve as useful biomarkers for frailty. Future research might be directed at better understanding the contribution(s) of various GeroMirs, in addition to miR-34a, that are released from senescent muscle, bone, and cartilage cells via EVs.

The research reviewed here suggests that targeting senescent cells and their EV-derived cargo may represent a potential therapeutic approach to treating age-related musculoskeletal dysfunction. Indeed, such an approach for removing senescent cells (senolytic therapy) or modifying their secretory profile (senomorphic therapy) has already shown potential for preventing joint degeneration and age-related bone loss. Other promising data suggest that additional small-molecule therapies may support musculoskeletal health by targeting microRNA components of the SASP. As noted earlier, ovariectomy in rodents induces bone loss and increases circulating SASP-associated miRNAs such as miR-34a; however, the observed bone loss and increase in SASP-associated miRNAs can be reduced with teriparatide or zolendronate treatment. Bone loss and increases in SASP-associated miRNAs may support musculoskeletal health by targeting microRNA substitutes for growth factors to enhance bone regeneration in vivo. Another potential strategy is to modify the increase in EV secretion that occurs in senescent cells. A recent high-throughput screen utilizing prostate cancer cells identified several existing small molecules that could be repurposed to either inhibit or stimulate exosome biogenesis. Future research might explore the repurposing of these drugs to prevent the release of EV-derived, SASP-associated miRNAs from senescent myotubes, chondrocytes, or osteocytes.

Another approach to preserve musculoskeletal function with age is to enhance the expression of endogenous antagonists to senescence-associated, EV-derived miRNAs. We have identified miR-34a as one of these SASP-related microRNAs in muscle, bone, and articular cartilage, whereas others have implicated miR-34a in age-related tissue dysfunction in the cardiovascular system and brain. Long noncoding RNAs (IncRNAs) can function as "sponges" for microRNAs, and thus have a role as competing endogenous RNAs (ceRNAs). The long noncoding RNA MALAT1 is a sponge for miR-34a but MALAT1 is depleted in senescent cells. MALAT1 itself appears to inhibit senescence, evident from experiments where MALAT1 siRNA increased senescent cell numbers in vitro. Importantly, MALAT1 decreases in patients with osteoarthritis and in patients with postmenopausal osteoporosis, and in mouse muscle atrophied with aging, hindlimb unloading, and glucocorticoid treatment (Fig. 3). Exogenous application of MALAT1 in EVs can reduce inflammation and joint degeneration in an experimental model of OA. Together these findings suggest that interventions to increase MALAT1 in multiple tissues may have beneficial effects by sponging miR-34a or suppressing its expression (Fig. 3). Of note, endurance and resistance exercise in older adults are associated with a significant increase in muscle MALAT1 expression. The studies reviewed here indicate that, although SASP factors carried by EVs are likely to play important roles in musculoskeletal diseases, several potential therapeutic approaches in development may be highly effective at inhibiting their activity to improve musculoskeletal health with aging.

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MCA and SF prepared the sections on osteoarthritis, AAS contributed the section on bone, and MWH prepared the introduction, the section on muscle, and the discussion.

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