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Concise Review

Cellular and Molecular Targets of Extracellular Vesicles from Mesenchymal Stem/Stromal Cells in Rheumatoid Arthritis

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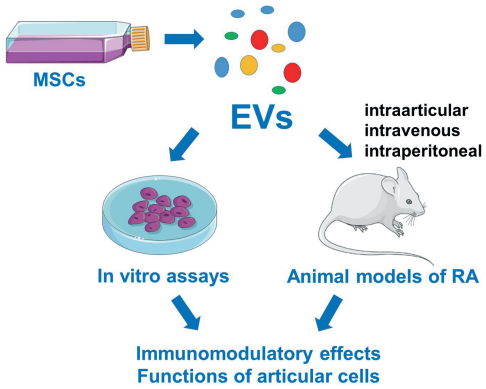
Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes progressive joint destruction. Despite the advances in the treatment of this condition there remains a clinical need for safe therapies leading to clinical remission. Mesenchymal stem/stromal cells (MSCs) play immunomodulatory and regenerative roles which can be partly mediated by their secretome. In recent years, the important contribution of extracellular vesicles (EVs) to MSC actions has received an increasing interest as a new therapeutic approach. We provide an extensive overview of the immunomodulatory properties of MSC EVs and their effects on articular cells such as fibroblast-like synoviocytes that play a central role in joint destruction. This review discusses the anti-arthritic effects of MSC EVs in vitro and in animal models of RA as well as their potential mechanisms. Recent preclinical data suggest that transfer of non-coding RNAs by MSC EVs regulates key signaling pathways involved in the pathogenesis of RA. We also examine a number of EV modifications for improving their anti-arthritic efficacy and carrier ability for drug delivery.

Key words: mesenchymal stem/stromal cells; extracellular vesicles; rheumatoid arthritis; fibroblast-like synoviocyte; experimental arthritis; non-coding RNAs.

Graphical Abstract



Significance Statement

We provide evidence supporting the immunomodulatory and joint protective actions of MSC EVs in vitro and in animal models of RA. Thus, MSC EVs represent an attractive strategy for future therapeutic applications due to the transfer of their active components or as a vehicle of drugs. Nevertheless, more preclinical studies are needed to address key questions such as molecular mechanisms responsible for their effects, routes of administration and dosification, tissue targeting, long-term effects and safety.

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune joint condition which can be considered as a clinical syndrome including several disease subsets.[1] Dysregulation of the immune system and activation of different inflammatory pathways result in persistent synovitis and damage to cartilage and bone. RA is characterized by the production of autoantibodies, predominance of pro-inflammatory cells and dysregulation of the peripheral immune tolerance. T cells, B cells, macrophages and dendritic cells infiltrate the synovium. In particular, hyperactive memory CD4+ T cells play a central role in early disease through several mechanisms such as their biased differentiation toward helper T cell (Th)1 and Th17 pathogenic T cells, cytokine production, activation of synovial cells and cooperation with B cells. Besides, aberrant activation of B cells contributes to the pathogenesis of RA through autoantibody production, antigen presentation and cytokine secretion.[2-4]

On the other hand, fibroblast-like synoviocytes (FLSs) undertake an aggressive proliferative transformation driving the invasion of articular cartilage and juxta-articular bone that leads to tissue destruction and perpetuation of disease.[5,6] Activated FLSs produce a wide range of pro-inflammatory mediators and catabolic enzymes such as matrix metalloproteinases (MMPs) as well as extracellular matrix components which play a main role in the establishment of chronic inflammation and joint damage.[7,8]

Although RA is incurable, early introduction of disease modifying anti-rheumatic drugs (DMARDs) of synthetic origin (conventional DMARDs and targeted DMARDs) and biological DMARDs can result in low disease activity (mild residual activity with low risk of damage progression) or even remission (no disease activity).[9] Despite the success of current therapies, there is still a need for novel therapeutic approaches as less than half of treated patients are in remission and approximately 40% of patients with refractory RA do not achieve the minimum acceptable target of American College of Rheumatology 20% (ACR20) response.[10,11]

Mesenchymal stem/stromal cells (MSCs) have a high therapeutic potential for autoimmune and inflammatory diseases. MSCs modulate the phenotype and functional properties of immune cells[12-14] and exert anti-arthritic effects in RA models.[15] Interestingly, some clinical trials have demonstrated that intravenous or intraarticular MSC therapy with autologous or allogeneic adipose tissue-derived MSCs (AD-MSCs), bone marrow-derived MSCs (BM-MSCs), umbilical cord-derived MSCs (UC-MSCs) or allogeneic multipotent progenitor cells, with or without DMARDs is safe in RA patients and exerts immunomodulatory and anti-inflammatory effects improving clinical symptoms of RA.[16-21] Of note, MSC treatments have been explored in challenging populations of patients with refractory RA. Administration of allogeneic UC-MSCs (intravenous route) with DMARDs induced a significant remission of disease according to the 28-joint Disease Activity Score (DAS28), and the Health Assessment Questionnaire. The therapeutic effects of a single-dose lasted 3-6 months and correlated with the increased percentage of Treg cells in peripheral blood.[22] This treatment with UC-MSCs showed a long-term efficacy (3 years).[18] In a randomized, placebo-controlled, phase Ib/IIa study, patients with a diagnosis of RA for ≥6 months and previous failure to at least 2 biologic DMARDs received 3 intravenous infusions of expanded allogeneic AD-MSCs on days 1, 8, and 15, with therapy assessment for 24 weeks. The results indicated an overall favorable safety profile of AD-MSCs besides a trend for clinical efficacy.[17] Taken together, these results suggest the interest of this approach although further research is needed to determine the most suitable patient profile to receive MSCs as well as the optimal treatment protocols.

A wide range of evidence has shown that MSC actions are mainly mediated by their secretome which includes soluble factors and extracellular vesicles (EVs).[23] These bilipid membrane vesicles have different composition and properties depending on cell phenotype and function or microenvironment conditions.[24] EVs with a diameter from 50 to 150 nm (small EVs, sEVs) derived from the inward budding of the endosomal membrane are usually called exosomes whereas microvesicles are EVs of 100 to 1000 nm in diameter (including medium-sized EVs and large EVs, lEVs) which are released by ectocytosis of the plasma membrane. Another example can be the vesicular apoptotic bodies (50-5000 nm range) which are released as part of the apoptotic process.[25] However, there are a number of limitations in EV studies as current methods do not allow to isolate pure EV subtypes and different EVs with overlapping size could be secreted by MSCs.[26] To address these issues, the International Society for Extracellular Vesicles works on the development of better isolation and characterization methods leading to appropriate standardization guidelines.[27]

EVs represent a mechanism of cellular communication in the microenvironment or at a distance. The content of EVs includes nucleic acids, lipids, proteins or mitochondria which can be transferred to recipient cells. In addition, surface proteins of EVs can interact with target cells via receptor-ligand binding. Specifically, the functional transfer of mRNAs or microRNAs (miRNAs) leads to the modulation of protein production and gene expression[28,29] while delivery of growth factors, cytokines, enzymes or lipid mediators has been shown to regulate a variety of functions in recipient cells.[30,31] Recent research has also demonstrated an improvement in mitochondrial dysfunction by miRNA transfer.[32] There is an increasing interest in the therapeutic applications of MSC EVs (mainly sEVs)[33] due to their immunomodulatory and regenerative properties. Different investigations have demonstrated that EVs released from MSCs are the main factors responsible for the activity of these cells[34] and the use of EVs may have some advantages compared with cellular treatments.[35] Therefore, EVs may represent a novel alternative to MSC-based therapy for the treatment of musculoskeletal diseases.[23] This review focuses on the ability of MSC EVs to regulate processes and pathways relevant to RA progression.

Immunomodulatory Effects of MSC EVs

EVs mediate the interactions between MSCs and immune cells. Recent research has shown that EV components such as RNAs and proteins are transferred to immune cells and can modulate their functions.[36,37] In this context, a number of studies have suggested that EVs can reproduce many of the anti-inflammatory and immunomodulatory effects of MSCs in vitro as well as in animal models of RA. Nevertheless, there are reports of a higher immunomodulatory ability of MSCs compared with their EVs which may be related to the presence of active molecules in the secretome and cell-to-cell contact.[38,39] In fact, the regulatory effects of mouse AD-MSCs

on some processes of the innate immune response are mainly mediated by the soluble fraction of their secretome.[40]

The immunomodulatory activity of MSC EVs has been supported by numerous studies showing their interactions with innate and adaptive immune cells (Fig. 1). BM-MSC EVs are shown to regulate the maturation and functions of human dendritic cells. The results of this study also suggest that transfer of miRNAs enclosed in EVs is a potential mechanism by which MSCs exert their modulatory role.[36] In addition, MSC EVs promote the polarization of monocytes/macrophages toward an anti-inflammatory phenotype and control the balance between pro- and anti-inflammatory cytokines. For example, an early study demonstrated that incubation of MSC EVs with THP1 cells or primary human or mice monocytes induces the M2 macrophage-like phenotype with elevated expression of anti-inflammatory interleukin(IL)-10 and transforming growth factor β1 (TGF-β1) and attenuated expression of pro-inflammatory (IL-1β, IL-6, tumor necrosis factor α [TNFα], and IL-12p40) genes.[41] In line with these results, mouse macrophages have been shown to uptake mouse AD-MSC EVs inducing the polarization into an anti-inflammatory and pro-resolving M2 phenotype.[42] Similarly, a mixed population of EVs (40 to 250 nm in diameter) from human AD-MSCs co-cultured with mouse macrophages, were internalized by these cells and induced cell proliferation and differentiation toward the M2 phenotype.[43]

Several reports have focused on the ability of MSC EVs to modulate both cell-mediated and humoral responses by affecting the differentiation, activation and functions of T and B cells. EVs from mouse BM-MSCs induce cell cycle arrest of T cells[44] while sEVs from primed human BM-MSCs promote immunosuppressive properties in resting MSCs towards T-cell proliferation.[45] In addition, EVs from human urine MSCs (U-MSCs) reduce T-cell proliferation in response to anti-CD3/CD28 stimulation.[46] EVs can also increase the apoptosis of T cells, as reported for mouse BM-MSC EVs.[47] Besides, BM-MSC EVs inhibit the activation of mouse splenocytes mediated by Toll-like receptor 4 (TLR4) and T-cell receptor leading to the inhibition of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and nuclear factor of activated T cells 1. These inhibitory effects of EVs may be mediated by TGF-β1 and pentraxin 3.[37]

MSC EVs promote the conversion of Th1 into Th2 cells and reduce the differentiation of Th17 cells.[48] Polarization of activated CD4+ T cells to CD4+CD25+FoxP3+ Treg cells by MSC EV treatment has also been reported in vitro and in animal models of RA.[41,47-51] In addition, co-culture of human UC-MSC sEVs with peripheral blood mononuclear cells (PBMCs) from RA patients, reduces Th17 cell numbers whereas Treg cells and TGF-β expression increase.[51]

An early report showed the inhibition of B cell differentiation and proliferation by human BM-MSC EVs in a CpG-stimulated PBMC co-culture.[52] Also, sEVs from human BM-MSCs reduced the proliferation of purified B cells and natural killer (NK) cells isolated from human PBMCs.[45] The inhibitory effect on B cells may be dependent on the regulation of the phosphatidylinositol 3-kinase-Akt signaling pathway and actin cytoskeleton.[53] In contrast, U-MSCs have shown B cell stimulating properties. These EVs contain interferon-γ (IFNγ), IL-6, CD40L and other molecules involved in B-cell functions.[46]

Concerning possible differences in the immunomodulatory properties of sEVs and lEVs, in vitro studies showed that sEVs and lEVs from mouse BM-MSCs exerted similar immunomodulatory actions although loss of activity after EV freezing was reported. Both fractions decreased the proliferation of concanavalin A-activated splenocytes, tended to reduce the percentage of CD8+IFNγ+ and increased CD4+IL10+

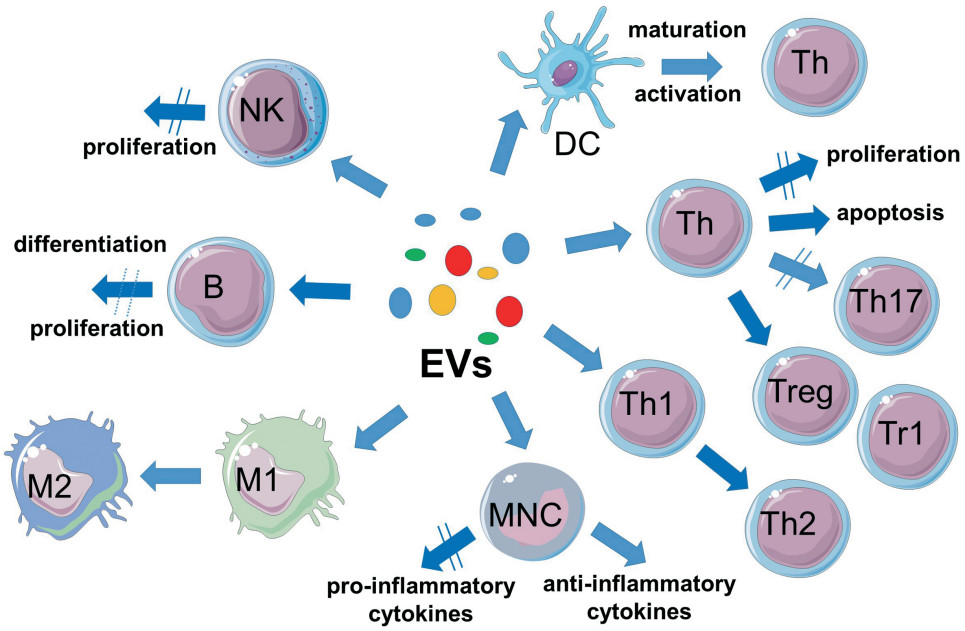

Figure 1. Immunomodulatory effects of MSC EVs. MSC EVs downregulate the differentiation and functions of cells driving the innate and adaptive immune responses whereas they promote the differentiation and functions of cells with regulatory activity. MSC EVs exert negative effects on DC maturation and activation as well as on the proliferation of Th, NK, and B cells. In addition, MSC EVs promote the conversion of Th1 into Th2 cells and reduce Th17 differentiation whereas the polarization to Treg cells, Tr1 cells, and M2 macrophages is increased. Abbreviations: B, B cell; DC, dendritic cell; EVs, extracellular vesicles; M, macrophage; MNC, monocyte; MSC, mesenchymal stem/stromal cell; NK, natural killer cell; Th, helper T cell; Treg, regulatory T cell; Tr1, type 1 regulatory T cell. (Some images were taken from Servier Medical Art: smart.servier.com).

type 1 regulatory (Tr1) cells and CD4+CD25+Foxp3+ Treg cells. Besides, they reduced the differentiation of B cells.[50]

MSC EV Treatments Regulate the Functions of Articular Cells

MSC EV internalization by FLSs has been demonstrated in vitro[54] and in animal models of inflammatory arthritis[55] leading to inhibitory effects in FLSs from RA patients or arthritic animals (Fig. 2). Thus, MSC EVs can regulate a number of FLS functions which are determinant for the progression of disease and tissue destruction, such as production of pro-inflammatory cytokines, angiogenic and catabolic mediators, cell proliferation, apoptosis resistance, migration and invasive ability.[56-58] For instance, BM-MSC sEVs inhibit the expression of CXC chemokine ligand 9 (CXCL9) which is highly expressed in RA FLSs and makes an important contribution to the activation, migration and invasive ability of these cells. Incubation of FLSs from RA patients with BM-MSC sEVs results in the transfer of miR-320a leading to the downregulation of CXCL9 and pro-inflammatory cytokines thus inhibiting FLS functions.[57]

In addition, BM-MSC EVs are internalized by human chondrocytes and decrease the induction of pro-inflammatory cytokines, cyclooxygenase-2, and collagenase by TNFα,[59] suggesting that MSC EVs may protect cartilage and bone in RA as reported in osteoarthritic cells.[60,61] Interestingly, MSC EVs exert inhibitory effects on chondrocyte apoptosis, pro-inflammatory mediators, degradative enzymes, and osteoclast differentiation[54,62] while promoting osteogenic differentiation and mineralization of MSCs.[63]

Administration of MSC EVs Controls the Progression of Experimental Arthritis

Recent evidence has demonstrated the anti-arthritic effects of MSC EVs in animal models of RA using mice, rats or pigs. These studies show that intraarticular, intravenous or intraperitoneal administration of MSC EVs reduces the immune response, joint inflammation and synovial hyperplasia as well as the degradation of articular cartilage and adjacent bone. Different EV populations may exhibit some differences in their anti-arthritic effects. As an example, lEVs from mouse BM-MSCs were less efficient than sEVs to ameliorate collagen-induced arthritis (CIA) in mice.[50]

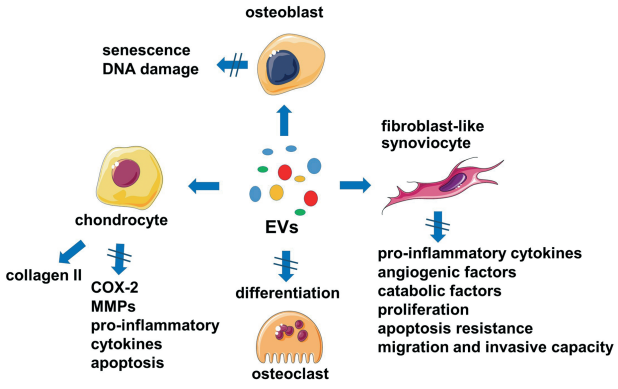

Figure 2. Potential beneficial effects of MSC EVs on RA articular cells. Abnormal activities of RA fibroblast-like synoviocytes can be inhibited by MSC EVs. In addition, they may exert protective effects on cartilage and bone through the downregulation of pro-inflammatory and catabolic mediators, senescence and DNA damage, and osteoclast differentiation. Abbreviations: COX-2, cyclooxygenase-2; EVs, extracellular vesicles; MMPs, matrix metalloproteinases; MSC, mesenchymal stem/stromal cell; RA, rheumatoid arthritis. (Some images were taken from Servier Medical Art: smart.servier.com.).

In an early report, EVs (mean diameter: 167 nm) from pig BM-MSCs administered by intraarticular route exerted anti-inflammatory effects and reduced TNFα gene expression in a model of antigen-induced synovitis in pigs. This treatment also decreased lymphocyte counts in synovial fluid and promoted some improvement in the quadruped gait suggesting pain reduction.[64] Other studies using rodent RA models also observed anti-arthritic efficacy of MSC EVs reproducing the effects of parent cells. For instance, an EV suspension (corresponding to 5.0×10^5 of human BM-MSCs) injected into the knee joint of antigen-induced arthritis mice significantly reduced paw swelling and histopathological changes through the suppression of Th17 polarization and synovial cellular infiltration.[65] In CIA rats, a single intravenous injection of sEVs from human UC-MSCs dose-dependently reduced joint inflammation, synovial hyperplasia and cartilage destruction. Besides, these EVs decreased T-cell proliferation, promoted the apoptosis of these cells and increased the Treg/Th17 ratio.[66] In the IL-1 receptor antagonist (IL-1Ra)$^{-/-}$ mouse, intravenous injection of EVs (mean diameter of 200 nm) isolated from mouse AD-MSCs reduced hind paw swelling, synovial hyperplasia, cartilage alterations, and serum levels of pro-inflammatory cytokines. These effects may be related to IL-1Ra transfer by EVs.[67]

Non-coding RNAs (ncRNAs) are components of EVs modulating a wide range of physiopathological processes. Although the exact mechanisms by which MSC EVs exert their anti-arthritic effects are not known, recent studies have suggested that miRNAs transferred by MSC EVs regulate relevant signaling pathways in animal models of RA (Supplementary Table S1) and in some cases, the downstream targets of these miRNAs have been identified.[54,56,57,62,68,69] For example, the anti-arthritic effects of human UC-MSC sEVs in CIA rats may be mediated by miR-140-3p. While the results of this study suggested that serum- and glucocorticoid-inducible kinase-1 (SGK1) is the target of miR-140-3p,[62] the relevance of this mechanism for RA is not clear as different studies have reported both pro-inflammatory[70,71] and anti-inflammatory roles of SGK1.[72]

Upregulation of mouse double minute 2 (MDM2) in RA FLSs blocks the function of p53 which acts as a negative regulator of NF-κB. The consequence is the activation of mitogen-activated protein kinases (MAPKs) and NF-κB inducing the expression of key pro-inflammatory and degradative mediators such as TNFα, IL-6, MMP-1, and MMP-13. MDM2 is the downstream target of miR-205-5p which is transferred by chondrogenic mouse BM-MSC sEVs to mouse FLSs in vitro and in the CIA model of RA.[68,73]

Krüppel-like factor 4 (KLF4) is an induced transcription factor that plays a major role in the progression of autoimmune arthritis by promoting FLS proliferation, MMP expression and secretion of pro-inflammatory cytokines.[74,75] The transfer of miR-21 may contribute to the anti-arthritic effect of mouse BM-MSC sEVs in the CIA model through the interaction with Ten Eleven Translocation 1 which demethylates KLF4 and promotes its expression in FLSs and bone tissue.[54]

Finally, another study reported the anti-inflammatory effects of human BM-MSC sEVs in the rat adjuvant arthritis

model via miR-34a transfer. This miRNA could directly bind to the 3′ UTR of cyclin I leading to the activation of the ataxia-telangiectasia, mutated (ATM)/ATM and Rad3-related protein/p53 signaling pathway. The consequences are the reduction of FLS proliferation, induction of FLS apoptosis and downregulation of IL-6, IL-8, and TNFα.[56]

Modifications of MSC EVs

There are many different factors influencing the composition and properties of MSC secretome, such as species, age and characteristics of donors, cell type, culture conditions, cell modifications, methods of isolation and purification of EVs, characterization, manipulation, preservation, etc. Therefore, EVs isolated from early-passage BM-MSCs are more immunosuppressive than those from late-passage cells while BM-MSCs in 3D cultures release more effective EVs compared with conventional 2D cultures.[37] Although recent research has suggested that normal culture conditions of MSCs are sufficient to isolate EVs with significant anti-arthritic activity in animal models,[65] different priming strategies have been used to improve their immunomodulatory properties and therapeutic potential. Pro-inflammatory priming induces important changes in the cargo of EVs and promotes the release of a higher percentage of sEVs compared to lEVs.[53,76] Thus, the immunomodulatory effects of sEVs from human BM-MSCs on purified B cells and NK cells are enhanced if parent cells are primed with pro-inflammatory cytokines (TNFα+IFNγ).[45] The age of the donor can influence the response to priming as the expression of RAB27B in primed adult AD-MSCs is lower than in pediatric AD-MSCs.[76] Pro-inflammatory priming induces the expression of immunomodulatory mediators such as TNFα-induced protein (TNFAIP) 6 and miRNAs which are secreted in sEVs.[45,77] In addition, stimulation of human UC-MSCs with lipopolysaccharide induces let-7b which regulates macrophage polarization into an anti-inflammatory M2 phenotype via TLR4/NF-κB/signal transducer and activator of transcription 3 (STAT3)/Akt signaling.[78]

Hypoxia pre-conditioning of human AD-MSCs also increases the content of miRNAs such as miR-223, miR-146b, miR-126, and miR-199a as well as the release of EVs.[43] On the other hand, heat shock of human AD-MSCs induces the expression of stress-response molecules which may enhance the anti-inflammatory effects of EVs.[79]

A number of reports have shown an improvement in the anti-arthritic effects of MSC EVs by forced overexpression of active ncRNAs. In vitro studies have demonstrated the efficacy of this strategy to regulate abnormal functions of RA FLSs such as increased proliferation, migration, invasion, production of pro-inflammatory mediators and apoptosis resistance. Some of these studies also showed inhibitory effects on angiogenesis. Therefore, sEVs from mouse BM-MSCs transfected with miR-150-5p were successfully uptaken by FLSs from RA patients leading to the inhibition of cell migration and invasion. These modified EVs also decreased tube formation by endothelial cells through the downregulation of MMP-14 and vascular endothelial growth factor (VEGF).[58] Similarly, EVs from human BM-MSCs overexpressing miR-124a inhibited cell proliferation and induced apoptosis in the MH7A cell line of RA FLSs.[80]

HAND2-AS1 is an example of a long ncRNA (lncRNA) target in RA. Transfer of this lncRNA by sEVs isolated from human BM-MSCs transfected with a HAND2-AS1 plasmid inhibits RA FLS abnormal functions through the regulation of miR-143-3p/TNFAIP 3/NF-κB.[81] The transfer of circular RNAs (circRNAs) through EVs has also been proposed as a new therapeutic approach to control inflammation and angiogenesis in RA. Therefore, EVs from human synovial MSCs stably overexpressing hsa_circ_0073244 (circEDIL3) inhibited VEGF production in RA FLSs which was mediated by the miR-485-3p/protein inhibitor of activated STAT3 (PIAS3)/STAT3 axis.[82] Recent studies show that EVs from human BM-MSCs can be excellent vehicles to transfer circFBXW7 to RA FLSs resulting in significant reductions in cell proliferation, migration, invasion and inflammation. This circRNA acts by sponging miR-216a-3p leading to histone deacetylase-4 (HDAC4) upregulation.[83]

Recent research has demonstrated that MSC EVs successfully deliver overexpressed ncRNAs to articular tissues in vivo to elicit anti-arthritic actions in animal models of RA (Supplementary Table S2).[55,58,82,83] For instance, administration of rat BM-MSC sEVs overexpressing miR-192-5p reduced the progression of arthritis in the rat CIA. Interestingly, this treatment reduced osteoclast numbers thus preventing bone loss. Another example is miR-192-5p which may act on synovial tissues through the downregulation of RAC2.[55] CircRNAs have also been successfully transferred to articular tissues in animal arthritis models. Some examples of anti-arthritic circRNAs are circEDIL3 which has PIAS3 as downstream target[82] and circFBXW7 which upregulates HDAC4 in synovial tissues confirming the in vitro results in RA FLSs.[83]

Other approaches have been proposed for EV applications in drug delivery. These modifications improve some properties of EVs such as a poor cell targeting or a short biological half-life after systemic administration, albeit with an important risk of altering their biological functions. Recently, a surface-editing strategy using the metabolic glycoengineering of human AD-MSCs led to the isolation of EVs (mean diameter 200 nm) targeting activated macrophages in inflamed joints. This strategy would reprogram the synovial microenvironment to promote the resolution of inflammation through M2 polarization and its efficacy was confirmed in CIA mice after intravenous administration of these EVs.[84] On the other hand, as epigenetic modifications of MSCs have been reported to improve their immunomodulatory properties in RA,[85] it is likely that this strategy may enhance the effects of EVs isolated from modified MSCs.

Concluding Remarks

RA is a chronic disease leading to progressive articular destruction and associated comorbidities. In recent years, advances in RA therapies have contributed to improve outcomes, but there remains a significant unmet clinical need. Despite the advances in the knowledge of RA pathogenesis, the complex mechanisms regulating key processes such as the loss of immunological tolerance, interactions between immune cells, synovial transformation or the transition from a pre-RA phase to established disease, are poorly understood. Thus, new efforts will be important to define the pathogenesis of RA, identify novel targets and individualize therapies.[6]

Various lines of evidence provide support for the immunomodulatory and joint protective actions of MSC EVs. In addition, EV-based therapeutics may have some advantages over cellular therapy as EVs do not have an endogenous tumorigenic potential, exert more predictable effects and are

easier to handle. Besides the possible therapeutic activity of endogenous EV components, the development of EVs as next-generation drug vehicles has attracted considerable interest[86] and different strategies to improve their therapeutic efficacy and/or carrier properties are being developed. Nevertheless, potential risks such as deleterious genetic or protein transfer, or undesirable immune alterations must be investigated.

In order to get reproducible results, more rigorous characterization of EVs following updated international guidelines is required to fully investigate their biological activity and therapeutic potential. In addition, all procedures for the production and application of EVs must follow strict protocols to avoid preparations with contaminants or differences in composition and activity.[25,33]

While MSC EVs have demonstrated efficacy in cultures of articular cells and in animal models of RA, we should take into account that preclinical models can only reproduce some aspects of this complex disease and thus inhibition of experimental arthritis should not be confounded with beneficial effects in RA. Although a number of clinical studies are testing the safety and efficacy of MSC EVs for a variety of inflammatory conditions, organ repair and osteoarthritis (https://clinicaltrials.gov), clinical trials for RA have not yet been reported. Before clinical translation, more well-designed preclinical studies are needed to address issues such as optimal doses, routes of administration, treatment frequencies, long-term effects and adverse effects.

Finally, to translate these results into successful therapies, further research is needed to improve large-scale production and isolation methods, quantification and characterization of EVs, determination of the cargo, pharmacokinetics, cell-targeting, cargo transfer mechanisms and safety profile.[33,87] Future RA clinical trials with MSC EVs will then evaluate whether this new strategy may be a vehicle of RA drugs or may act by itself, likely as a complement of other RA therapies. In the next few years we will be able to definitively answer the question as to whether MSC EVs are a feasible approach for RA treatment.

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Conflict of Interest

The authors declared no potential conflicts of interest.

Author Contributions

M.J.A. wrote the manuscript. M.I.G. contributed to material collection, design of figures and revision. All authors approved the final version of the manuscript.

Data Availability

No new data were generated in support of this research.

Supplementary Material

Supplementary material is available at *Stem Cells Translational Medicine* online.

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