Therapeutic mesenchymal stem cells in rheumatic diseases: rationale, clinical data and perspectives

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Abstract

Mesenchymal stem or stromal cells (MSCs) are easily isolated from bone marrow or fat tissue and their potential of multilineage differentiation has initially led to the development of strategies for tissue engineering applications. More recently, they have gained much interest based on their trophic and immunomodulatory properties that have stimulated their evaluation in various clinical trials aiming at modulating the host immune response in graft-versus-host disease or autoimmune diseases. The clinical applications of MSCs for rheumatic diseases are limited and address primarily their potential to help tissue repair/regeneration. The aim of the present review is to focus on the mechanisms by which MSCs might exhibit a therapeutic potential in rheumatology and present the current data on the undergoing clinical trials. Special attention is given to miRNA expression in rheumatic pathologies and their possible modulation for future innovative strategies as biomarkers or therapeutic targets.

Keywords: mesenchymal stem cells, osteoarthritis, rheumatoid arthritis, immunosuppresssion, miRNA, cartilage repair, regeneration, cell therapy

Introduction

Mesenchymal stem cells or multipotent mesenchymal stromal cells (MSC) are adult stem cells exhibiting characteristic properties that make them promising candidates for cell-based clinical therapies. Historically, their capacity of multilineage differentiation has been explored in a number of strategies for skeletal tissue regeneration [1]. More recently, these cells have been shown to exhibit immunosuppressive and healing capacities, to improve angiogenesis and prevent apoptosis or fibrosis through paracrine mechanisms. This has opened the way for novel therapeutic applications for the treatment of inflammatory and degenerative rheumatic diseases including rheumatoid arthritis (RA), osteoarthritis (OA) as well as bone and cartilage genetic disorders. Although most of the data are pre-clinical results, some clinical applications have been initiated that primarily address the potential of MSCs for skeletal tissue repair. More understanding on the mechanisms regulating the therapeutic efficacy of MSCs has been achieved but further improvement is needed before their use for therapeutic applications in rheumatic diseases may be generalized.

Rheumatic diseases: pathogenesis and treatments

Rheumatic diseases are characterized by symptoms involving the musculoskeletal system, primarily the joints but also muscles and, extending sometimes to the deeper organs, as the heart. Osteoarthritis (OA) is the most common form of rheumatic disorders affecting cartilage, synovium, muscle and subchondral bone. OA affects 40% of people>70 years of age and its prevalence increases with age and other risk factors such as obesity, skeletal malformations, mechanical stress and genetic factors [2]. Current therapeutic approaches are largely palliative aiming at reducing symptoms. Widely used therapies including nonsteroidal anti-inflammatory drugs (NSAIDs), cyclo-oxygenase 2 inhibitors, hyaluronic acid and glucosamine are moderately effective and leave patients with substantial pain [3]. Other treatment options called disease-

modifying OA drugs (DMOADs) include a wide array of agents such as chondroitin sulphate, matrix metalloproteinase inhibitors, calcitonin and avocado-soybean unsaponifiables and are currently under clinical evaluation. The potential clinical benefit of DMOADs is to slow or halt disease progression and even reverse disease progression but to date; none have convincingly demonstrated clinically meaningful effects. Future therapeutic development should consider the complexity of OA to both improve symtoms and address the issue of disease modification.

Rheumatoid arthritis (RA) is a chronic autoimmune disease and the most common inflammatory arthritis (0.5-1% of the adult population worldwide). A variety of antigens (toll-like receptor agonists, bacterial DNA, type II collagen, rheumatoid factor, cyclic citrullinated pepetides,…) has been proposed to lead to T and B cell activation. In addition to inflammation, hyperplasia of the synovium infiltrated by macrophages, B and $CD4^+$ T cells results in secretion of degradative enzymes leading to cartilage destruction [4]. The disease is associated to genetic susceptibility with higher prevalence and greater severity of RA in patients who express the human leukocyte antigen (HLA)-DR1, -DR4 or -DR14 alleles. Many disease-modifying anti-rheumatic drugs (DMARD) are available but methotrexate is usually the first line treatment. For the patients who do not respond to these treatments, the use of biological agents (tumour necrosis factor (TNF)-α inhibitors, IL-1RA, anti-CD20 or anti-IL-6R antibodies) is the next step [5]. However, about one-third of patients with active RA do not respond well to DMARD or a first TNF inhibitor treatment. For those patients, the use of a second biological agent may offer some benefit, but there remain uncertainties with regard to the magnitude of treatment effects suggesting that a better evaluation of the treatment at the biological level or the development of alternative therapeutic approaches are needed.

Among the other most prevalent rheumatic diseases, spondyloarthropathies, in particular ankylosing spondylitis (AS) and psoriatic arthritis (PsA) affect respectively, approximately 0.5% of white Europeans and about 0.1-0.5% of the population. The main symptoms of AS are spinal pain (due to inflammation, bone erosion and spur formation) and progressive ankylosis in some

patients [6]. The major causative factors of AS are genetic, with the gene encoding HLA-B27 being the most important genetic factor [7]. PsA is characterized by inflammation of peripheral joints, skin and nails, spine, entheses and dactylitis. PsA has also been associated with genetic susceptibility. Traditional systemic therapies as well as a number of biological treatments, especially the inhibitors of TNFα, have demonstrated significant benefit for both spondyloarthropathies and the ability to control damage [8]. However, a key aspect of treatment is accurate diagnosis and assessment, which allow the institution of appropriate treatment in a timely fashion.

Although the advent of biotherapies has revolutionized patient care in rheumatology, there still exists unmet need for a number of patients who do not respond to anti-inflammatory treatments and for patients with degenerative OA. Novel pharmacologic therapeutic interventions are being developed but alternative approaches based on stem cell therapies need to be evaluated.

Properties of mesenchymal stem cells

MSCs are the stem cells of the musculoskeletal tissue leading to the formation of cartilage, bone, tendon, ligament, muscle and adipose tissue. They can be isolated from a variety of tissues including bone marrow (BM), adipose tissue, synovium, periosteum, umbilical cord vein or placenta [9]. MSCs are defined by their capacity to adhere to plastic, their phenotype: $CD73^+$, $CD90^+$, $CD105^+$, $CD11b^-$, $CD19^-$, $CD45^-$, HLA-DR^- and $CD34^-$ (or $CD34^+$ when isolated from adipose tissue) and their trilineage differentiation potential [10]. In addition, these cells exhibit immunoregulatory properties (for review, see [11]) and secrete a variety of soluble mediators that are crucial for cell proliferation or survival. These key properties make these cells attractive for tissue regeneration or repair in various clinical applications and particularly in rheumatology.

Immunomodulatory properties of mesenchymal stem cells

The capacity of MSCs to modulate the immune response is well documented (for review, see [11]). The immunosuppressive activity of MSCs is not constitutive and needs to be elicited or "activated" by the pro-inflammatory signals, interferon (IFN)-γ and tumor necrosis factor (TNF)-α, IL-1α, IL-1β or toll-like receptor (TLR) ligands [12,13]. Upon activation, MSCs release a variety of immunosuppressive factors that will suppress immune cell proliferation in response to various stimuli. Indeed, it has been reported that MSC inhibit T cell proliferation through induced anergy and cell cycle arrest at the G1 phase [14]. MSCs have also been reported to inhibit B cell proliferation and function [15]. However, contradictory data exist since Traggiai reported enhanced proliferation and differentiation of memory B-cells toward plasma cells [16]. Whilst there is agreement on the ability of MSCs to inhibit natural killer (NK) cell proliferation, their influence on NK cell-mediated cytotoxicity is also controversial. Besides their effects on lymphocytes and NK cells, MSCs suppress the generation of dendritic cells (DC) from monocytes or progenitor cells isolated from bone marrow and inhibit their maturation and function [17,18]. Finally, it was shown recently that MSCs inhibit Th17 cell differentiation and induce fully differentiated Th17 cells to exert a T cell regulatory phenotype [19].

The underlying mechanisms of MSC-mediated antiproliferative effect are likely to act through the concomitant secretion of several factors. Among the factors that have been described, the key immunomodulators include indoleamine 2,3-dioxygenase (IDO), nitric oxyde (NO) prostaglandin E2 (PGE2), human leukocyte antigen (HLA)-G5, transforming growth factor (TGF)-β1 and heme oxygenase (HO)-1. The role of these molecules is likely to be complementary and/or partial. As an example, the effect of IDO is prominent in human MSCs, whereas NO seems to play a major role in murine MSCs. Furthermore, MSCs may act differently depending on the inflammatory status of the environment. Indeed, depending on TLR stimulation, TLR4-primed MSCs, or MSC1, mostly elaborate pro-inflammatory mediators, while, TLR3-primed MSCs, or MSC2, express mostly immunosuppressive ones [13]. The

immunomodulatory properties of MSCs may thus be of interest to modulate the immune response in patients with inflammatory rheumatic diseases such as RA, AS or PsA.

Paracrine activity of mesenchymal stem cells

Besides the secretion of immunosuppressive factors, MSCs produce a variety of other soluble factors. These include cytokines, chemokines, growth factors that exhibit diverse functions. Historically, MSCs were identified in the bone marrow as fibroblastic stromal cells supporting haematopoiesis through the secretion of various cytokines and growth factors, such as stem cell factor (SCF), interleukin (IL)-6, lymphocyte inhibitory factor (LIF), granulocyte macrophage-colony stimulating factor (GM-CSF), G-CSF or M-CSF [20]. Since the expansion of the MSC-based research activity, other biological functions have been attributed to MSCs. Indeed, they exhibit a pro-angiogenic activity, primarily via the secretion of hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) which is one of the most potent factors [21,22,23]. They also exert anti-fibrotic, anti-apoptotic and proliferative properties [24]. HGF or adrenomedullin have been suggested to be involved in the anti-fibrotic function of MSCs as well as matrix metalloproteinases (MMPs) and tissue inhibitors of MMP (TIMPs) [25,26], while SDF-1 and Sfrp2 have been identified as anti-apoptotic factors [27,28]. The combination of the different functional roles of secreted factors may be of interest for joint tissue regeneration both by stimulating the proliferation of endogenous progenitor cells and preventing the more differentiated phenotypes from apoptosis or dedifferentiation that may occur in degenerative disorders.

Differentiation potential of mesenchymal stem cells

A large body of literature is available on the differentiation process of MSCs from various tissue origins toward chondrocytes, adipocytes, osteoblasts and cells of the musculoskeletal system, namely tendinocytes, ligamentocytes and vascular smooth muscle cells. Although controversial,

MSCs have been reported to transdifferentiate into cells from non mesoderm-origin, including cardiomyocytes, hepatocytes or neurons [29,30]. While MSC transdifferentiation has been shown in several in vitro studies, transdifferentiation of MSCs in vivo is limited and a low number of MSCs has been shown to participate in the regeneration of specific tissues such as heart. This raises a point about the range of plasticity of MSCs. It is noteworthy to highlight that a number of signaling pathways seems to be activated in proliferating BM-MSCs suggesting a pre-programming of these cells towards the chondrocytic, osteoblastic, adipocytic and smooth myocytic lineages [31]. This last study supports the notion of lineage-priming and further argues for the use of BM-MSCs for the cell therapy of skeletal disorders.

MSC-based therapies in clinical rheumatology

Immunomodulation of inflammatory arthritis

The remarkable potential of MSCs to modulate the host immune response, mainly by inhibiting the proliferation of T lymphocytes, introduced the possibility that they might be effective in inflammatory arthritis where the T cell response is prominent. Studies using the collagen-induced arthritis (CIA) experimental mouse model reported improvement of clinical and biological scores after injection of MSCs derived from BM or adipose tissue [32,33]. Contradictory results are however reported (for review [34].. More recently, our group has shown that IL-6-dependent PGE2 secretion by primary murine MSCs inhibits local inflammation in experimental arthritis in a time-dependent fashion which may explain the discrepancies observed between studies [35].

Although clinical trials involving the use of MSCs for the treatment of inflammatory autoimmune diseases such as Crohn disease or diabetes, are underway, none has been conducted for RA treatment. Some years ago, hematopoietic stem cell transplantation (HSCT) was conducted in patients with refractory RA who were randomised to receive unmodified bone marrow transplantation, containing hematopoietic as well as stromal cells, or CD34-selected

HSCT [36]. An ACR70 response was attained in 27.7% of the 18 patients who had received CD34-selected cells and 53.3% of the 15 who had received unmanipulated cells but did not reach statistical significance. The results of this trial as well as discrepancies between studies in pre-clinical animal models suggest that a therapeutic effect of MSCs may depend on the inflammatory status of the receiver at the time of cell administration.

Stimulation of endogenous regeneration in degenerative arthritis

In degenerative arthritis, MSC-based therapy may stimulate cartilage regeneration by endogenous progenitors or prevent tissue degradation through the secretion of bioactive factors. Indeed, transplantation of autologous MSCs to caprine joints subjected to total meniscectomy and resection of the anterior cruciate ligament resulted in regeneration of meniscal tissue and significant chondroprotection [37].

In humans, eight clinical trials are currently recruiting patients to test the safety and efficacy of MSC injection for OA treatment. A phase I/II trial is currently evaluating the effect of MSC injection with hyaluronan (in the form of Chondrogen^{TM}) to prevent subsequent OA in patients undergoing meniscectomy. Clinical data available on the commercial website of the company (Osiris Therapeutics Inc) indicate that MSC administration significantly reduced pain and degenerative lesions associated with OA. Pain scores improved from six months to one year following treatment. The mechanism of MSC-based therapy remains unknown but it has been speculated that secreted biofactors might reduce fibrocartilage formation or decrease degradation by inhibiting proteinases. Moreover, although OA is not considered an inflammatory disease, secretion of cytokines, namely IL-1β and TNF-α, and immune responses may also be suppressed thanks to the immunomodulatory effects of MSCs. The various reports therefore argue for a therapeutic efficacy of MSCs to prevent or limit OA lesions in patients.

Tissue engineering for large defects in late stage arthritis

The limited repair capacity of articular cartilage and the absence of pharmacological agents able to stimulate cartilage regeneration have led to the development of novel approaches of cartilage repair as an alternative to the surgical methods currently used. In particular, the third generation of autologous chondrocyte implantation (ACI) was reported to improve clinical symptoms and the quality of the repaired tissue. Moreover, associated to microfracture, ACI was shown to lead to better clinical outcomes compared with osteochondral grafts [38,39].

The number of reports on MSC transplantation for cartilage repair in human is limited. However, the feasibility of bone marrow-derived MSC (BM-MSC) implantation for cartilage repair has been tested several years ago in few patients with various outcomes but generally, an improvement of clinical symptoms and formation of hyaline cartilage in some areas were observed [40,41,42,43]. A more recent study using BM-MSCs transplanted on platelet rich-fribrin glue in full-thickness cartilage defects resulted in similar outcomes [44]. Although the number of patients was low is this pilot study, all symptoms improved and magnetic resonance imaging revealed complete fill of large-sized defects (average: 5.8 cm^2). Moreover, the efficacy of BM-MSC implantation was recently reported by comparison to ACI in 72 matched patients [45]. The conclusion of this study was that BM-MSC implantation is as effective as chondrocytes for cartilage repair and required less knee surgery, reduced costs and minimized donor-site morbidity.

Safety of MSC-based therapies

The great potential of MSC-based therapies for different clinical applications has raised questions on the safety of MSC infusions in patients. A large body of evidence suggests that MSCs are recruited into tumors where they can deliver a variety of agents, including angiogenic factors, chemokines and growth factors. However, many studies have reported contradictoring results (for review, see [46]). While some investigators report that MSCs inhibit tumor growth,

others report that MSCs promote tumors. In tumors, MSCs may alter the behavior of the cancer cells and may also differentiate to carcinoma-associated fibroblasts (CAF), which are known to be involved in cancer progression [47]. It has also been reported that MSCs may undergo spontaneous transformation in vitro and form tumors in vivo [48,49]. However, the investigators have since retracted because they found that the transformed MSCs were contaminated with tumor cell lines. On the contrary, the inhibitory effect of MSCs on tumor growth has been shown in a number of studies [50,51]. This discrepancy may be explained by several experimental differences such as the dose of MSCs or the animal model used. More importantly, the timing of injection may be a critical element. The injection of MSCs into established tumors result in tumor growth inhibition whereas coinjection of MSCs and tumor cells yield to tumor promotion.

In addition, it has been reported that MSCs are not fully immunoprivileged and show low persistence in vivo further arguing about low risks of adverse effects. Of importance, no evidence of tumor formation has been reported so far in over 1,000 patients treated with MSCs for a variety of indications.

New concepts and future therapeutic perspectives in osteo-articular diseases

Although there is large evidence that miRNAs are involved in organogenesis and cell differentiation, a limited number of studies focus on miRNA expression in MSCs (for review, see [52]). However, the possible regulatory effects of some miRNAs on the differentiation of MSCs towards the main skeletal lineages, osteoblasts, adipocytes and chondrocytes have been described [53]. While the demonstration that modulation of miRNAs might lead to stable differentiated phenotypes is lacking, tissue engineering approaches might benefit from a better understanding of the regulatory pathways influencing MSC differentiation towards a specific lineage.

While novel therapeutic approaches, pharmaceutic- or cell-based therapies, are being developed, there is a critical need for tools that might have utility for early diagnosis, prognosis and even

treatment. Identification of biomarkers might therefore help early diagnostic between rheumatic diseases that are heterogeneous but share common features. Biomarkers may also be used as prognostic tools to monitor the progression and evaluate the severity of the disease. Maybe more importantly, they may help predict the response of patients to a particular treatment and guide praticians' therapeutic options. Indeed, aberrantly expressed microRNAs (miRNAs) have considerable potential for use as biomarkers in rheumatology. MicroRNAs are small, noncoding ribonucleic acids (RNAs) that play critical roles in the regulation of host genome expression at the posttranscriptional level. During the last past years, miRNAs have emerged as key regulators of various biological processes including cell lineage commitment, differentiation, maturation, and maintenance of homeostasis. Thus, it is not surprising that dysregulated miRNA expression profiles have been documented in a broad range of diseases such as cancer, inflammatory and autoimmune diseases including RA and OA [54]. Moreover, the presence and stability of miRNAs in body fluids provide fingerprints that can serve as molecular biomarkers for disease diagnosis and therapeutic response.

MicroRNAs in OA

Two studies using miRNA microarray large-scale analysis have initially described altered miRNA expression in OA cartilage [55,56]. They reported, respectively, 76 and 17 dysregulated miRNAs between OA and normal cartilage. These studies highlighted the fact that miRNAs might be implicated in OA pathogenesis albeit no common dysregulated miRNAs was shown. A recent study reported the overexpression of miR-146a in low grade OA cartilage in comparison with healthy cartilage whereas its expression decreased with the severity of OA [57]. The levels of expression of miR-146a were inversely correlated with the increase of MMP13 but the proof that MMP13 is a direct target of miR-146a was not shown. MMP13 was shown to be regulated by another miRNA, miR-27b. Its expression was downregulated by IL-1β treatment in OA chondrocytes and correlated with the increase of MMP13 levels [58]. Finally, IL-1β treatment

was reported to induce miR-34a expression in rat chondrocytes and down-regulation of type II collagen and iNOS as well as reduction of apoptotic cells [59].

The role of only one miRNA has been functionally validated in vivo in OA pathogenesis. Indeed, the role of miR-140 dysregulation in OA cartilage was then reported after generation of a mouse line through a targeted deletion of miR-140 [60]. The knockout of miR-140 predisposed to age-related OA, whereas overexpression of miR-140 in chondrocytes protected from OA. The authors showed that miR-140 was necessary to maintain low levels of the metalloproteinase ADAMTS5, which is a critical proteinase in OA pathology, and thus maintain homeostasis. They also reported that transfection of chondrocytes with miR-140 downregulated Il-1β-induced ADAMTS5 expression [61]. Indeed, loss of miR-140 contributes to OA-like changes but these studies also demonstrate a role in cartilage development and homeostasis.

MicroRNAs in RA

Over the past 3 years, the abnormal expression of dozen miRNAs has been reported in patients with RA, both in the circulation and within the rheumatoid inflamed joints. Most of them are up-regulated: miR-16, miR-132, miR-133a, miR-142-3p, miR-142-5p, miR-146a, miR-155, miR-203, miR-223; and only 3 are reported under-expressed: miR-124a, miR-363 and miR-498a [62]. Importantly, none of these 12 miRNAs are specific for RA. As miR-146a and miR-155 are involved in the development of innate and adaptative immune cells and finely tune immune and inflammatory responses, not surprisingly they were the first and most studied miRNAs in RA samples [63,64,65]. And they are the only one having their role investigated in vivo in mouse models of RA so far. The expression of miR-146a and miR-155 is induced by proinflammatory conditions such as IL-1, TNF and TLRs. Mice deficient for miR-155 are protected from CIA and systemic over-expression of miR-146a in CIA mice, prevents joint destruction but had no effect on inflammation [66,67]. It is also not surprising that miR-16 and miR-223 were both reported in RA as they are among the most abundant miRNAs expressed in the blood under normal

conditions. Their higher expression levels in RA might only reflect increased cellularity, a systemic enrichment of specific hematopoietic lineages in the blood from RA patients. In steady state conditions, miR-16 is ubiquitously expressed at high levels while miR-223 is considered as a hematopoietic-specific miRNA with crucial functions in myeloid lineage development. It is reported that miR-223 is the only miRNA markedly up-regulated in peripheral naive CD4+ T-lymphocytes from RA patients compared with healthy donors [68]. Most of the miRNAs abnormally expressed in RA tissues are involved in hematopoietic-related cancers and have been reported as dysregulated in other types of immune-mediated inflammatory disorders, thus appearing not so much specific for a RA-specific pathogenic process, but rather reflecting a loss of homeostatic regulation of inflammatory events.

Detection and high stability of miRNAs in the serum and plasma is possible because they circulate within microparticles that render them resistant to drastic conditions. Indeed, plasma or serum miRNAs open great opportunities for a novel type of biomarker molecules. Although the 12 miRNAs identified so far in RA cannot be used as diagnostic biomarkers for RA patients as they are not disease-specific, several of them have been suggested for monitoring disease activity. Murata et al. showed that plasma levels of miR-16, miR-146a, miR-155 and miR-223 are inversely correlated with disease activity [69]. These miRNAs are also detectable in the synovial fluid, and their expression levels can discriminate patients with RA and OA, but data comparing with other rheumatisms and healthy donors are missing. However, no correlation exists between plasma and synovial fluid miRNA levels. The miRNAs detected in synovial fluid and plasma have different origins, the expression pattern of synovial fluid miRNAs, but not the one from plasma, being similar to the miRNAs secreted by the synovial tissue.

Finally, since miRNAs are highly effective and specific regulators of gene expression, they are attractive agents for the development of innovative therapeutic strategies. There is so far only one publication reporting data supporting the therapeutic potential of a miRNA-based treatment in RA, showing that the enforced expression of miR-15a into the knee joint of autoantibody-

mediated arthritic mice is able to induce synovial membrane cell apoptosis by negatively regulating the local expression of Bcl-2 [57]. But no clinical data support this strategy design as valuable for treating RA. In terms of clinical application, the use of miRNA-based therapeutics has anyway to overcome major drawbacks, mainly targeted delivery and safety issues, before being considered as a realistic option.

MicroRNAs in mesenchymal stem cells

Expression and role of miRNAs in MSCs has been recently reviewed [70]. The regulatory function of miRNAs on the differentiation of MSCs towards the main skeletal lineages, osteoblasts, adipocytes and chondrocytes is the subject of major investigations. However, to our knowledge, the role of miRNAs in other important functions of MSCs such as secretion of trophic or immunomodulator mediators has not been described so far. There is cumulating data suggesting that MSCs secrete microparticles containing characteristic proteins or miRNAs that may serve as a new way of cell-to-cell communication [71,72]. Although speculative, these results pave the way to the hypothesis that MSCs might exert miRNA-mediated biological effects on other cells through secretion of miRNA. These effects might explain at least in part, the trophic action of MSCs though the secretion of mediators (proteins but also miRNAs) that might regulate different pathogenic processes in rheumatic diseases.

Conclusion and future perspective

MSC-based cell therapies represent innovative strategies for the treatment of forms of rheumatic diseases for which currently available treatments are limited. Encouraged by the results on pre-clinical studies, feasibility as well as safety of MSC administration are currently being investigated in phase I/II clinical trials for cartilage defects following degenerative arthritis and the therapeutic potential of the procedures are under evaluation for various applications. MSC-

based therapies will with no doubt benefit of the current trials as well as the elucidation/better understanding of the mechanisms by which MSCs promote tissue repair.

The therapeutic application of miRNAs represents a promising approach in rheumatic diseases. Because miRNAs are highly dysregulated during OA or RA pathogenesis, they are promising candidates as biomarkers or therapeutic targets. The clinical application of miRNAs will however require a better understanding of their function within the context of diseases before being used as either diagnostic markers or therapeutic targets. Likewise, delivery of proteins or miRNAs by MSCs may be one important way to reprogram tissue-injured cells and mediate cell-cycle re-entry, thus favoring tissue regeneration. However, much remains to be elucidated. Future studies will definitely be necessary for better understanding the biology of MSCs and facilitating the development of novel MSC-based therapeutic approaches for rheumatic diseases.

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Executive summary

- Rheumatic diseases affect the joints causing primarily lesions to cartilage, which lead to functional alterations and represent an important source of handicap worldwide.
- Current treatment options include the use of disease-modifying drugs and biotherapies, essentially TNF inhibitors, but a number of patients do not respond to these treatments.
- Mesenchymal stem cells are promising candidates for cell-based therapies of rheumatic diseases thanks to their differentiation potential towards chondrocytes and osteoblasts as well as their immunosuppressive and trophic properties.
- The safety and therapeutic efficacy of mesenchymal stem cell is being evaluated in the clinics to induce cartilage repair or endogenous regeneration.
- Future studies will be necessary to validate the role of MSC-based therapies in rheumatic pathologies and develop new tools, in particular miRNA-based therapeutics, for better diagnosis, prognostic and therapeutic response.

Bibliography

* of interest

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1. Vinatier C, Bouffi C, Merceron C *et al*. Cartilage engineering: Towards a biomaterial-assisted mesenchymal stem cell therapy. *Curr. Stem Cell Res. Ther.* 4,318-329 (2009).
2. Valdes AM, Spector TD. Genetic epidemiology of hip and knee osteoarthritis. *Nat. Rev. Rheumatol.* 7,23-32
3. Hunter DJ. Pharmacologic therapy for osteoarthritis--the era of disease modification. *Nat. Rev. Rheumatol.* 7,13-22
4. Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 423,356-361 (2003).
5. Klarenbeek NB, Kerstens PJ, Huizinga TW, Dijkmans BA, Allaart CF. Recent advances in the management of rheumatoid arthritis. *Bmj* 341,c6942
6. Toussirot E. Late-onset ankylosing spondylitis and spondylarthritis: an update on clinical manifestations, differential diagnosis and pharmacological therapies. *Drugs Aging* 27,523-531
7. Thomas GP, Brown MA. Genetics and genomics of ankylosing spondylitis. *Immunol. Rev*. 233,162-180
8. Mease PJ. Psoriatic arthritis: update on pathophysiology, assessment and management. *Ann. Rheum. Dis.* 70 Suppl 1,i77-84

9. Chen Y, Shao JZ, Xiang LX, Dong XJ, Zhang GR. Mesenchymal stem cells: a promising candidate in regenerative medicine. *Int. .J Biochem. Cell Biol.* 40,815-820 (2008).
10. Dominici M, Le Blanc K, Mueller I *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8,315-317 (2006).
11. Ghannam S, Bouffi C, Djouad F, Jorgensen C, Noel D. Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. *Stem Cell Res. Ther*. 1,2 (2010).
12. Ren G, Zhang L, Zhao X *et al*. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2,141-150 (2008).
* Demonstration of MSC priming by IFN, IL-1 and TNF for immunomodulatory activity
13. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM. A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. *PLoS One* 5,e10088 (2010).
14. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood* 105,2821-2827 (2005).
15. Corcione A, Benvenuto F, Ferretti E *et al*. Human mesenchymal stem cells modulate B-cell functions. *Blood* 107,367-372 (2006).
16. Traggiai E, Volpi S, Schena F *et al*. Bone marrow-derived mesenchymal stem cells induce both polyclonal expansion and differentiation of B cells isolated from healthy donors and systemic lupus erythematosus patients. *Stem Cells* 26,562-569 (2008).
17. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE. Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. *J. Immunol.*.177,2080-2087 (2006).
* Demonstration of the role of MSC on generation and function of dendritic cells
18. Djouad F, Charbonnier LM, Bouffi C *et al*. Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. *Stem Cells* 25,2025-2032 (2007).
19. Ghannam S, Pene J, Torcy-Moquet G, Jorgensen C, Yssel H. Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype. *J. Immunol.* 185,302-312 (2010).
20. Ringden O, Le Blanc K. Allogeneic hematopoietic stem cell transplantation: state of the art and new perspectives. *Apmis* 113,813-830 (2005).
21. Rehman J, Traktuev D, Li J *et al*. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 109,1292-1298 (2004).
22. Kinnaird T, Stabile E, Burnett MS *et al*. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ. Res.* 94,678-685 (2004).
23. Nguyen BK, Maltais S, Perrault LP *et al*. Improved function and myocardial repair of infarcted heart by intracoronary injection of mesenchymal stem cell-derived growth factors. *J. Cardiovasc. Transl. Res.*.3,547-558 (2010).
24. Mias C, Lairez O, Trouche E *et al*. Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction. *Stem Cells* 27,2734-2743 (2009).
25. Li L, Zhang Y, Li Y *et al*. Mesenchymal stem cell transplantation attenuates cardiac fibrosis associated with isoproterenol-induced global heart failure. *Transpl. Int.* 21,1181-1189 (2008).
26. Huang HI, Chen SK, Ling QD *et al*. Multilineage Differentiation Potential of Fibroblast-Like Stromal Cells Derived from Human Skin. *Tissue Eng. Part A* (2009).

27. Wang F, Yasuhara T, Shingo T *et al*. Intravenous administration of mesenchymal stem cells exerts therapeutic effects on parkinsonian model of rats: focusing on neuroprotective effects of stromal cell-derived factor-1alpha. *BMC Neurosci*..11,52 (2010).
28. Mirotsou M, Zhang Z, Deb A *et al*. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell-released paracrine factor mediating myocardial survival and repair. *Proc. Natl. Acad. Sci. U S A* 104,1643-1648 (2007).
* Important function of MSC for survival and repair in a model of infraction
29. Jiang Y, Jahagirdar BN, Reinhardt RL *et al*. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418,41-49. (2002).
30. Tropel P, Platet N, Platel JC *et al*. Functional neuronal differentiation of bone marrow-derived mesenchymal stem cells. *Stem Cells* 24,2868-2876 (2006).
31. Delorme B, Ringe J, Pontikoglou C *et al*. Specific Lineage-Priming of Bone Marrow Mesenchymal Stem Cells Provides the Molecular Framework for Their Plasticity. *Stem Cells* 27,1142-1151 (2009).
** Interesting study on the plasticity and lineage-priming of MSC
32. Augello A, Tasso R, Negrini SM, Cancedda R, Pennesi G. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. *Arthritis Rheum.* 56,1175-1186 (2007).
33. Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D, Delgado M. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. *Gastroenterology* 136,978-989 (2009).
34. Schurgers E, Kelchtermans H, Mitera T, Geboes L, Matthys P. Discrepancy between the in vitro and in vivo effects of murine mesenchymal stem cells on T-cell proliferation and collagen-induced arthritis. *Arthritis Res. Ther.* 12,R31 (2010).
35. Bouffi C, Bony C, Courties G, Jorgensen C, Noel D. IL-6-dependent PGE2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis. *PLoS One* 5,e14247 (2010).
36. Moore J, Brooks P, Milliken S *et al*. A pilot randomized trial comparing CD34-selected versus unmanipulated hemopoietic stem cell transplantation for severe, refractory rheumatoid arthritis. *Arthritis Rheum.* 46,2301-2309 (2002).
37. Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum.* 48,3464-3474 (2003).
* First demonstration of paracrine effect of MSC on cartilage regeneration
38. Vavken P, Samartzis D. Effectiveness of autologous chondrocyte implantation in cartilage repair of the knee: a systematic review of controlled trials. *Osteoarthritis Cartilage* 18,857-863 (2010).
39. Brittberg M. Cell carriers as the next generation of cell therapy for cartilage repair: a review of the matrix-induced autologous chondrocyte implantation procedure. *Am. J. Sports Med.* 38,1259-1271 (2010).
40. Wakitani S, Imoto K, Yamamoto T *et al*. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 10,199-206 (2002).
41. Wakitani S, Mitsuoka T, Nakamura N *et al*. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. *Cell Transplant.* 13,595-600 (2004).
42. Wakitani S, Nawata M, Tensho K *et al*. Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. *J. Tissue Eng. Regen. Med.* 1,74-79 (2007).
43. Kuroda R, Ishida K, Matsumoto T *et al*. Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthritis Cartilage* 15,226-231 (2007).

44. Haleem AM, Singergy AA, Sabry D *et al*. The Clinical Use of Human Culture-Expanded Autologous Bone Marrow Mesenchymal Stem Cells Transplanted on Platelet-Rich Fibrin Glue in the Treatment of Articular Cartilage Defects: A Pilot Study and Preliminary Results. *Cartilage* 1,253-261 (2010).
45. Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am. J. Sports Med*. 38,1110-1116 (2010).
46. Klopp AH, Gupta A, Spaeth E, Andreeff M, Marini F, 3rd. Concise review: Dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? *Stem Cells* 29,11-19 (2011).
** Comprehensive and recent review on the tumoriginity of MSC
47. Mishra PJ, Mishra PJ, Humeniuk R *et al*. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. *Cancer Res.* 68,4331-4339 (2008).
48. Rubio D, Garcia-Castro J, Martin MC *et al*. Spontaneous human adult stem cell transformation. *Cancer Res.* 65,3035-3039 (2005).
49. Rosland GV, Svendsen A, Torsvik A *et al*. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. *Cancer Res.* 69,5331-5339 (2009).
50. Cousin B, Ravet E, Poglio S *et al*. Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both in vitro and in vivo. *PLoS One* 4,e6278 (2009).
51. Zhu Y, Sun Z, Han Q *et al*. Human mesenchymal stem cells inhibit cancer cell proliferation by secreting DKK-1. *Leukemia* 23,925-933 (2009).
52. Lakshmipathy U, Hart RP. Concise review: MicroRNA expression in multipotent mesenchymal stromal cells. *Stem Cells* 26,356-363 (2008).
53. Guo L, Zhao RC, Wu Y. The role of microRNAs in self-renewal and differentiation of mesenchymal stem cells. *Exp. Hematol.*
54. Alevizos I, Illei GG. MicroRNAs as biomarkers in rheumatic diseases. *Nat. Rev. Rheumatol.* 6,391-398
55. Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A. Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One* 3,e3740 (2008).
56. Jones SW, Watkins G, Le Good N *et al*. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13. *Osteoarthritis Cartilage* 17,464-472 (2009).
57. Nagata Y, Nakasa T, Mochizuki Y *et al*. Induction of apoptosis in the synovium of mice with autoantibody-mediated arthritis by the intraarticular injection of double-stranded MicroRNA-15a. *Arthritis Rheum.* 60,2677-2683 (2009).
58. Akhtar N, Rasheed Z, Ramamurthy S *et al*. MicroRNA-27b regulates the expression of matrix metalloproteinase 13 in human osteoarthritis chondrocytes. *Arthritis Rheum.* 62,1361-1371
59. Abouheif MM, Nakasa T, Shibuya H *et al*. Silencing microRNA-34a inhibits chondrocyte apoptosis in a rat osteoarthritis model in vitro. *Rheumatology (Oxford)* 49,2054-2060
60. Miyaki S, Sato T, Inoue A *et al*. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. *Genes Dev.* 24,1173-1185
* Important study on the role of miRNAs in osteoarthritis disease
61. Miyaki S, Nakasa T, Otsuki S *et al*. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. *Arthritis Rheum.* 60,2723-2730 (2009).
62. Duroux-Richard I, Presumey J, Courties G *et al*. MicroRNAs as new player in rheumatoid arthritis. *Joint Bone Spine* 78,17-22

63. Nakasa T, Miyaki S, Okubo A *et al*. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. *Arthritis Rheum.* 58,1284-1292 (2008).
64. Pauley KM, Satoh M, Chan AL *et al*. Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. *Arthritis Res. Ther.* 10,R101 (2008).
65. Stanczyk J, Pedrioli DM, Brentano F *et al*. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. *Arthritis Rheum.* 58,1001-1009 (2008).
66. Bluml S, Bonelli M, Niederreiter B *et al*. Essential role for micro-RNA 155 in the pathogenesis of autoimmune arthritis. *Arthritis Rheum.* 63,1281-1288 (2011).
67. Nakasa T, Shibuya H, Nagata Y, Niimoto T, Ochi M. The inhibitory effect of microRNA-146 expression on bone destruction in arthritis. *Arthritis Rheum.* 63,1582-1590 (2011).
68. Fulci V, Scappucci G, Sebastiani GD *et al*. miR-223 is overexpressed in T-lymphocytes of patients affected by rheumatoid arthritis. *Hum. Immunol.* 71,206-211 (2010).
69. Murata K, Yoshitomi H, Tanida S *et al*. Plasma and synovial fluid microRNAs as potential biomarkers of rheumatoid arthritis and osteoarthritis. *Arthritis Res. Ther*. 12,R86 (2010).
70. Zou Z, Zhang Y, Hao L *et al*. More insight into mesenchymal stem cells and their effects inside the body. *Expert Opin. Biol. Ther.* 10,215-230 (2010).
71. Bruno S, Grange C, Deregibus MC *et al*. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *J. Am. Soc. Nephrol.* 20,1053-1067 (2009).
72. Collino F, Deregibus MC, Bruno S *et al*. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs. *PLoS One* 5,e11803