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Review

Mesenchymal stem cells for the management of inflammation in osteoarthritis: state of the art and perspectives

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SUMMARY

Osteoarthritis (OA) is the most common form of degenerative arthritis, mainly characterized by the degradation of articular cartilage and associated with subchondral bone lesions. Novel therapeutic approaches for OA include cell-based therapies that have become thriving areas of research and development. In this context, mesenchymal stem or stromal cells (MSCs) have gained much interest based on their trophic and immunomodulatory properties that can help tissue repair/regeneration. The present review article discusses the interest of using MSCs in cell-therapy approaches with a focus on the mechanisms by which MSCs might exhibit a therapeutic potential in OA. Special attention is given to the anti-inflammatory function of MSCs and on miRNA modulation in OA for possible future innovative strategies. The paper also presents the current data on the undergoing MSCs-based clinical trials in OA.

Introduction

Osteoarthritis (OA), the most prevalent form of arthritis, affects up to 15% of the adult population and is principally characterized by degradation of the articular cartilage of the joint, associated with subchondral bone lesions. Chronic, low-grade inflammation contributes to symptoms and disease progression. Networks of diverse innate inflammatory danger signals, including chemokines, cytokines and alarmins are activated in OA. Besides inflammatory mediators, biomechanical injury and oxidative stress compromise the viability of chondrocytes, leading to hypertrophic differentiation and pro-catabolic responses with further extracellular matrix (ECM) degradation. Better understanding the inflammatory pathophysiology should help identifying different OA subtypes in the population and should lead to the development of new therapeutic options.

OA is one of the most prevalent diseases of the elderly and is a top cause of disability. There are few treatment options for OA patients and most of them aim at reducing pain and controlling inflammation to improve function. Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroid injections are largely used since many years but the current treatment strategies have no impact on the progressive degeneration of joint tissues[1,2]. Recent studies suggest that disease-modifying treatments are possible. Similar to the approach that has been successful for rheumatoid arthritis (RA), biotherapies targeting inflammatory mediators such as TNF-α, IL1 or IL6 have been tested. Although these strategies led to a majority of disappointing results[3–5], some biotherapies are still under evaluation. As an example, we would like to point out a recent study using adalimumb (a humanized monoclonal antibody targeting TNFα) that reports statistically significant less erosive evolution on the radiological image in erosive hand OA patients with clinical joint swelling[6]. The current data indeed suggest that co-inhibition of several pro-inflammatory cytokines may be more efficient in OA[7]. In this context, mesenchymal stromal/stem cell (MSC)-based therapy seems attractive because this innovative therapeutic strategy could provide an enlarged anti-inflammatory potential. MSCs are immunosuppressive cells, which can decrease inflammation through the release of anti-inflammatory factors (including IL1RA) and decrease monocyte activation. In this review, we summarize recent data confirming the role of MSCs as a potential therapeutic strategy in OA.

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The role of inflammation in OA

Although OA has generally been proposed as a degenerative disease, recent work suggested that low-grade inflammatory processes could promote disease symptoms and accelerate disease progression[8]. Some of the cartilage matrix catabolic products probably activate macrophages and other innate immune cells to release inflammatory cytokines, which in turn promote cartilage damage progression by altering chondrocyte function[9]. The interplay between the immune system and cartilage is not well understood but evidence of regulation of acute-phase response signaling pathway, the complement pathway, and the coagulation pathway in the joint fluid of OA patients has been reported, suggesting a contribution of inflammation to joint damage[10].

GWAS and studies of familial clusters and twins have also shown a relation of OA susceptibility with inflammation; the influence of genetic factors being close to 70%. Studies of candidate genes and genome analysis have identified polymorphisms or mutations in genes involved in the synthesis of ECM or the signaling pathways of inflammation. Among the identified genes are *ADAMTS-12*, cartilage intermediate layer protein (*CILP*), vitamin D receptor (*VDR*), cyclooxygenase *(COX)2*, asporin (*ASPN*), Growth and Differentiation Factor (*GDF*)*5*, IL4 receptor. The polymorphism rs20417 in the promoter of the *COX2* gene contributes to the genetic risk for hip and knee OA[11]. However a correlation with the expression level of PGE2 in the synovial fluid has not been demonstrated.

Synovial membranes from patients with OA demonstrate low grade synovitis compared to RA but with high expression of cytokines. OA synovial tissue shows an increase in immune cell infiltrates associated with pro-inflammatory cytokine expression, including tumor necrosis factor (TNF)α, IL1β, IL6, IL8 and IL22. Moreover, activation of the innate immune system contributes to the persistence of OA synovial low-grade inflammation. Damage to cells and cartilage ECM resulting from repeated microtrauma and senescence generates damage-associated molecular patterns (DAMPs) that activate the innate immune system through the toll-like receptor (TLR) pathway[12]. DAMPs include fragments generated from ECM degradation such as proteoglycans, intracellular proteins such as heat-shock proteins or DNA. By inducing the release of Alarmins (high mobility group box protein 1 S100A8 and S100A9) by monocytes, they contribute to the inflammatory cascade. The inflammatory process activates the release of enzymes by chondrocytes and monocytes resulting in enhanced catabolic process. These enzymes include proteins of A Disintegrin And Metalloproteinase with Thrombospondin motifs (ADAMTS) family and matrix metalloproteinases (MMP)1, 3, 13, which are directly responsible of ECM remodeling. It has also been shown that the joint synovial fluid from OA patients contains a small number of MSCs but their role in OA pathogenesis or cartilage regeneration has yet to be established[13]. OA is therefore an inflammatory musculoskeletal disease involving both innate and adaptive immune response as shown by high levels of pro-inflammatory cytokines and downstream target factors.

Characteristics and properties of mesenchymal stem cells

Mesenchymal stromal or stem cells (MSCs) can be isolated from a variety of adult or neonatal tissues, primarily bone marrow, fat tissue, dental pulp, placenta or umbilical cord. They are characterized by their fibroblastic shape, their immunophenotype ($CD11b^-$, $CD14^-$, $CD34^-$, $CD45^-$, HLA-DR^-, $CD73^+$, $CD90^+$, $CD105^+$) and their trilineage potential of differentiation towards bone, cartilage and adipose tissue[14]. Endogenous MSCs have been proposed to localize in a perisinusoidal location in the bone marrow[15] and to be marked by nestin or leptin-receptor[16,17] in mice or CD146 in humans[18]. But perisinusoidal cells do not display all the properties of MSCs suggesting that another skeletal stem cell should exist. Indeed, two studies have very recently reported the identification of endogenous mouse skeletal stem cell (mSSC). The first one identified osteo-chondroreticular stem cells in the bone marrow on the basis of Gremlin 1 expression while the other identified a subpopulation of stem cells that generates two multipotent progenitor cell types giving raise to bone, cartilage and stromal tissue[19,20].

MSCs exert different functions thanks to a variety of secreted factors. They produce growth factors, such as transforming growth factor (TGF)β, hepatocyte growth factor (HGF), basic fibroblast growth factor (FGF) or vascular endothelial growth factor (VEGF), that induce proliferation and angiogenesis of various cell types, in particular fibroblasts, epithelial or endothelial cells. Another important property of MSCs is their capacity to rescue cells from apoptosis induced by trauma, oxidative environment, radiation or chemical injury. Some key proteins have been proposed to play such role. Insulin growth factor (IGF)1, interleukin (IL)6 and stanniocalcin-1 are essential for apoptotic reversal in fibroblasts while VEGF, HGF and TGFβ1 have been shown to protect endothelial cells from apoptosis[21,22]. The anti-fibrotic effect of MSCs has been largely shown *in vitro* and in different pre-clinical models of fibrosis (for review, see Ref. 23). Although it has been argued that MSCs might exert profibrotic function, there is no example from the literature that shows that MSC transplantation induces fibrosis on a developing or established disease. The protective effect of MSCs extends beyond anti-fibrosis to reduction of scar tissue formation as exemplified in a recent review of the literature[24].

Finally, maybe the most studied property of MSCs is their anti-inflammatory and immunosuppressive role on cells from the adaptive and innate immune responses. MSCs interact with T cells and inhibit the proliferation and differentiation of naïve T lymphocytes towards the Th1 or Th17 phenotype. We also demonstrated that repolarization of Th17 cells depends on PD-L1 expression on MSCs[25]. The inhibition of differentiation of naïve T lymphocytes was associated with an increase in the number of functional natural Treg cells and enhanced IL-10 secretion. However, MSCs were not able to generate Treg cells when cultured with mature Th1 or Th17 lymphocytes[26]. In parallel, MSCs induce a Th2-like immune response, independently of T regulatory cell generation[27]. The immunomodulatory effect of MSCs is not specific, and primary skin fibroblasts are able to inhibit an inflammatory immune response, as efficiently as MSCs. Similar to MSCs, skin fibroblasts secreted nitric oxide (NO), IL6, prostaglandin (PG)E2 and induced a Th2-like immune response[28]. The secretion of PGE2 induced by IL6 plays an important role in this immunomodulatory effect[27].

Both soluble and contact-dependent signals from the environment trigger the therapeutic effect of MSCs, which in turn, accordingly respond via the secretion of various mediators. The soluble factors are released in the extracellular environment at the vicinity of the cells or entrapped into extracellular vesicles (EVs), which can transfer their content from one cell to another over long distances and have been isolated from virtually all body fluids[29]. In studies on tissue regeneration, injection of MSC-derived EVs has been shown to improve at least one major/clinical parameter associated with organ dysfunction[30]. Although the effect of MSC-derived EVs has not been addressed in rheumatic diseases, it may be speculated that they may improve the outcomes of OA or RA[31].

The choice of MSC source for efficient therapeutic effect

Since the identification of MSCs as regulators of the immune response in the late 1990's, the concept that MSCs are immune

privileged cells has been proposed[32]. This has stimulated research using major histocompatibility (MHC)-unmatched allogeneic cells in several clinical applications. For osteo-articular diseases, the use of allogeneic MSCs or MSCs from human origin was reported to be efficient in reducing the clinical signs of collagen-induced arthritis[33–35] or in improving OA in murine models without the need of immunosuppressive drugs addition (for review, see[36]). However, several preclinical and clinical studies have pointed out that allogeneic cells may elicit a humoral and cellular immune response *in vivo* and harbor the risk of inducing MHC specific reactivity[37,38]. While the use of allogeneic MSCs has to face significant challenges, the therapy using autologous MSCs may raise several difficulties. In addition to the expansion time required for producing sufficient quantities of cells, the variable potency of MSCs between patients and the need for suitable quantities of MSCs in acute conditions may limit the use of autologous MSCs in some clinical applications[39]. Half of the clinical trials relied on the use of autologous cells but the efficacy of autologous over allogeneic MSCs-based therapy still needs to be demonstrated. Because the therapeutic effect of MSCs is proposed to be due to a hit-and-run mechanism, the rapid elimination of allogeneic MSCs may not be a problem, even though we may assume that MSC therapy may gain by prolonging the persistence of the cells.

Another mean of enhancing MSC therapy could be to pre-activate the cells before injection. Pre-activation of MSCs by inflammatory mediators was evaluated in the murine model of acute respiratory distress syndrome[40]. It resulted in higher protective capacity which was associated with increased expression of IL10 and IL1RA (receptor antagonist), reduction of the lung injury score, lower pulmonary edema and reduced accumulation of bronchoalveolar lavage inflammatory cells and cytokines compared with non activated cells. However contradictory results are available. MSCs pre-activation with IFN-γ failed to prolong allograft survival in a model of rat corneal allograft survival[41]. In rheumatic diseases, pre-activation of MSCs with IFN-γ and TNF-α failed to ameliorate established arthritis[42]. The inflammatory environment encountered by MSCs upon injection is likely sufficient to activate their anti-inflammatory function.

A better appreciation of the tissue origin of MSCs as well as the heterogeneity of MSC subpopulations within a tissue is of importance for optimizing their therapeutic efficacy for specific disease targets. MSCs isolated from bone marrow or synovial tissue have higher chondrogenic differentiation potential that those isolated from other tissues while higher adipogenic activity was demonstrated in synovium- and adipose-derived cells[43]. While the differentiation potential of MSCs may vary from source to source, the age of the donor as well as the health status may influence their therapeutic effectiveness in certain diseases[44]. Indeed, MSCs from healthy donors and OA patients present similar colony forming unit-fibroblast (CFU-F) capacity but a loss of proliferative activity related with age[45]. MSCs isolated from patients with end stage OA are functionally deficient in terms of their *in vitro* proliferation and differentiation potential[46]. These data suggest that MSCs from OA patients have become senescent and that a correlation between the proliferative potential and the age of native MSCs is suggested[36]. On the other hand, specific markers for human MSC subsets are lacking and most of the procedures used for MSC expansion under Good Laboratory Practices (GLP) rely on plastic adherence and give rise to heterogeneous cell populations. There is evidence that MSCs change their properties according to different culture conditions and in response to different tissue environments[47]. Moreover, culture-expanded MSCs have been reported to lose their trophic function[48,49]. Indeed, potency assays must be established and standardized to ensure that patients will receive functional MSCs and comparable doses of cells.

Understanding the molecular mechanisms associated with the therapeutic effect of MSCs in OA

The interest of using MSCs in stem cell therapies for cartilage regeneration in osteoarticular diseases has been largely discussed[31,50,51]. They have been used in tissue engineering approaches where they can be associated with a scaffold and implanted in cartilage lesions. Clinical evidence supports the notion that MSCs may be an effective treatment for traumatic injury in chondral and osteochondral cartilage defects but few studies report the interest of MSC-based tissue engineering approaches in OA[52]. In one study focusing on patients with OA of the knee, equivalent clinical outcomes were observed with patients receiving MSC- or cell-free scaffolds but better arthroscopic and histological scores were shown in the cell-transplanted group[53]. However, evidence that MSCs could be better than chondrocytes is still lacking and an easier and more direct approach could be the injection of MSCs without scaffold[36,54]. Indeed, MSCs have also been evaluated as paracrine factors-releasing cell therapy products after local or systemic injection (for review see Ref. 31). Through the secretion of mediators, which may stimulate endogenous regeneration and proliferation of tissue progenitors or, counteract apoptosis or cartilage degeneration, they may contribute to cartilage repair/protection.

The proliferation of chondrocytes has been shown to be stimulated by coculture with bone marrow- or synovium-derived MSCs[55,56]. In a coculture model where human OA chondrocytes were incubated with adipose-derived MSCs (ASCs), we were also able to demonstrate a reduction in the expression of hypertrophic, fibrotic and inflammatory markers[57,58]. The anti-fibrotic effect was mainly attributed to the secretion of HGF by ASCs[58]. In this system, ASCs alone produced very low levels of pro-inflammatory cytokines and chemokines but they significantly decreased the secretion of IL6, IL8, monocyte chemoattractant protein (MCP)1 and macrophage inflammatory protein (MIP)1α of both chondrocytes and synoviocytes[57].

In addition to their anti-inflammatory potential and their capacity to stimulate endogenous cartilage regeneration, MSCs could differentiate *in vivo* and replace injured cartilage[59]. However, few studies have investigated the immunosuppressive potential of differentiated MSCs towards chondrocytes[60]. Although one study reported that differentiated MSCs retained their ability to suppress allogeneic immune responses[61], other reports indicated that MSC differentiation resulted in the loss of their immunosuppressive properties[62,63]. Differentiated MSCs were shown to secrete lower levels of PGE2 and NO, two important mediators of MSC-based immunosuppression, and to express higher levels of major histocompatibility component (MHC)-I, MHC-II, CD80 and CD86[63]. These findings suggest that chondrogenically differentiated MSCs not only may lose *in vivo* their immunosuppressive potential but also promote the proliferation and activation of T lymphocytes. The mechanisms by which MSCs could regenerate cartilage in OA are not elucidated but whether their ability to differentiate into chondrocytes may impact their capacity to inhibit inflammatory responses *in vivo* needs further investigation.

The regenerative potential of MSCs was confirmed *in vivo* using experimental OA models. Intra-articular injection of murine ASCs reduced the histological lesions of cartilage degradation in the experimental model of collagenase-induced OA (CIOA) when injected in a preventive protocol[64]. Moreover, the therapeutic effect was significant in this inflammatory CIOA model while no effect of ASC treatment on cartilage destruction, osteophyte formation or chondrogenesis in ligaments was found in the destabilization of median meniscus (DMM) model[65]. In the CIOA model, lower levels of S100A8, S100A9 alarmins and IL1β were detected few hours after

ASC injection suggesting that ASCs reduced macrophage activation. Indeed, efficacy of ASC injection was observed in the model with high activation of the synovial membrane and therefore correlated with their anti-inflammatory property. In a rabbit model, Desando *et al.* demonstrated that intra-articular injection of ASCs had a structural benefit. ASC treatment inhibited the progression of OA, and was associated with a significant decrease of Laverty's score at 16 weeks compared to the controls[66]. A decreased expression of TNF-α and MMP-1 was observed in the ASC-treated groups at 16 and 24 weeks. In the low dose group (2×10^6 cells/joint), the reduction of MMPs and TNF-α expression in menisci and synovial membrane was more effective than in the high dose (6×10^6 cells/joint). Several other studies reported the effect of MSCs or ASCs on cartilage protection and OA prevention in different models of OA[67–69]. Indeed, MSCs are not only involved in the maintenance of joint homeostasis but may be of interest to restore or protect against inflammation or degenerative changes associated with OA progression.

Role of microRNAs (miRNAs) in the molecular mechanisms sustaining MSC functions

miRNAs are small non-coding endogenous RNAs with the capacity to modulate the expression of multiple protein-encoding genes at the posttranscriptional level. MicroRNAs control a huge number of biologic functions such as proliferation, apoptosis or differentiation[70]. In MSCs, the function of more than 60 miRNAs has been described in a recent review article[71]. Most of them have been shown to be involved in differentiation and proliferation. Indeed, global miRNA disruption through Drosha and Dicer knockdown (both are essential component for biogenesis of miRNAs) resulted in significantly reduced potential of differentiation of human MSCs[72]. In chondrocytes, Dicer knockdown induced a decreased proliferation and accelerated differentiation towards a hypertrophic phenotype[73]. Several miRNAs including miR-23b, -29a, -140, -194, -199 and -574-3p have been shown to regulate the differentiation of MSCs into chondrocytes[74–79]. In addition, miRNAs have been found to function in migration or apoptosis of MSCs. More recently, the role of miRNAs in the paracrine effect of MSCs has been exemplified.

Various recent papers highlighted the importance of miRNAs in controlling the immunosuppressive function of MSCs. As an example, miR-27b knockdown had a positive influence on the allosuppressive activity that inhibits T-cell proliferation via inverse correlation of CXCL12 expression in cultured ASCs[80]. MiR-181a regulated the proliferation of MSCs through TGF-β signaling pathway and MSC immunosuppressive properties through the MAPK signaling pathway. Specifically, miR-181a enhanced IL-6, VEGF, and indoleamine 2,3-dioxygenase (IDO) expression, resulting in attenuation of the MSC immunosuppressive properties *in vitro* and *in vivo*[81]. Up-regulation of miR-155 reduced the immunosuppressive capacity of MSCs by repressing iNOS expression[82]. In addition, correction of the diabetic wound-healing impairment with MSC treatment was associated with a significantly increased expression of miR-146a and related down-regulation of its target pro-inflammatory genes[83]. Conversely, Matysiak *et al.* have identified miR-146a as a negative regulator of BM-MSC immunosuppressive function via targeting PGE2 secretion[84].

Validation of new miRNAs in this process could have implications in basic science but also potentially in clinical research if the modulation of the expression of one miRNA can enhance the immunosuppressive effect of MSCs. Indeed, up- or down-regulation of the expression of some miRNAs may represent a new interesting strategy in stem cell-based therapy in OA. Over-expression of miR-140 may have a regulatory role in modulating cartilage homeostasis and OA development through the inhibition of several OA-related genes, such as ADAMTS5[85]. MiR-145 is another potential candidate because it up-regulates the expression of genes, such as collagen II and miRNAs, such as miR-140 and miR-655, which play important roles in cartilage[86]. A complementary strategy is to use miRNAs able to inhibit or prevent OA-associated inflammation. Mir-146 and miR-15a have been shown to reduce inflammation and degradation initiated by IL1β and reduce synovial hyperplasia in RA, respectively[87,88]. However, additional work will be necessary to determine the optimal procedure to improve stem cell technology for the treatment of OA.

Deregulation of microRNAs in OA

The altered expression of several miRNAs in OA cartilage has initially been described in two different studies although no common miRNA was reported[89,90]. Overexpression of miR-22 in normal chondrocytes resulted in an increased expression of IL1β and MMP13 and a decreased expression of Aggrecan. Inhibition of miR-22 in OA chondrocytes blocked the inflammatory processes by inhibiting IL1β and MMP13[89]. Other studies described the overexpression of miR-146a, miR-9 and miR-34a, which regulate TNF-α or MMP13, suggesting that they may have a protective role in OA[88,91]. A more recent study has showed differential expression of seven novel miRNAs in OA and normal chondrocytes whose function still need to be validated[92].

IL1β is one of the major cytokine responsible for cartilage degradation in OA and in a previous study, we have shown that miR-24 is repressed in IL1β-treated chondrocytes and in cartilage of OA patients[93]. MiR-146a has been proposed to negatively regulate MMP13 although its expression gradually decreases with advancement of the disease[94]. The expression of miR-146a was inversely correlated with the expression of MMP-13 and was strongly induced after chondrocyte stimulation with IL1β[87]. MiR-146a was reported to be a negative regulator of the inflammatory response and it could also be a negative regulator of MMP13 in osteoarthritic cartilage. MiR-140 is a critical miRNA in OA as it plays important role in chondrogenesis and cartilage development[85,95]. *In vivo* knockout of miR-140 predisposed to age-related OA while overexpression of miR-140 protected mice from OA through the modulation of MMP13 and ADAMTS5 expression. More recently, the importance of miR-125b, miR-127-5p, miR-148a and miR-21 in OA development and progression has been described[96]. Finally, Beyer and co-authors identified a signature of circulating microRNAs differentially expressed in OA[97]. Three miRNAs, let-7e, miR-454 and miR-885-5p were identified as predictors for severe knee or hip OA. Let-7e was the most promising OA biomarker candidate since it was associated with a higher susceptibility to get more than one joint replacement surgery independently of age, sex or body mass index.

All of these data highlight the utmost importance of miRNAs in MSC homeostasis. Deregulation of miRNAs in OA patients seems critical since they impact the inflammatory environment as well as the functional properties of MSCs, in particular their differentiation and immunosuppressive potential. Modulation of individual miRNAs in MSCs is therefore a promising strategy to enhance the therapeutic efficacy of MSCs[98].

Application of MSCs to cell therapy for OA patients

Despite encouraging pre-clinical data, only few preliminary clinical studies on the use of autologous stem cells have been published for articular cartilage damaging diseases. Actually, the original clinical studies focused on the use of MSCs for cartilage

repair with in mind the observation that articular cartilage has to be repaired to prevent subsequent OA changes. Most clinical studies concerned knee joint injuries[99–101] while one study was on ankle cartilage defect[102]. Wakitani and collaborators injected autologous BM-MSCs embedded in a collagen gel directly into the articular cartilage defect of osteoarthritic knee joints[53]. Twelve patients received autologous bone marrow cell transplants, and twelve were cell-free controls. A better arthroscopic and histological score was observed in the cell-transplanted group even though no clinical improvement was demonstrated after 6 months. Another non-randomized study compared 36 patients with autologous chondrocyte implantation and 36 patients with autologous BM-MSCs. After 2 years, similar outcomes were obtained for the two procedures but the autologous BM-MSC-based approach was safer and less expensive[99]. A recent study compared the safety of chondrocytes vs MSC implantation. Neither tumors nor infections were observed on a mean 75 months of follow-up[103]. All these studies generally reported presence of a hyaline-like cartilage repair tissue within the primitive cartilage defects.

In OA, no randomized studies have been performed yet. Two studies reporting the use of autologous BM-MSCs for treating a small number of patients with moderate-to-severe knee OA were recently published by Iranian groups[104,105]. Absence of side effects was reported after 1-year follow-up together with an improvement in walking time and reduction in walking pain. Moreover, MRI displayed an increase of cartilage thickness and a decrease in the size of subchondral edemas in half of patients[105]. Another non-controlled clinical trial has shown that local injection of ASCs improved clinical symptoms of pain and WOMAC index[106] and in a dose-escalation study, up to 100 millions of cells were well tolerated[107]. A last report on 12 patients who received 40×10^6 autologous BM-MSCs into the knee joint revealed improvement of cartilage morphology and quality using MRI T2 mapping suggesting a possible structural benefit of stem cell therapy[108]. Finally, our recent results from a phase I dose escalation study on 18 patients with knee OA showed safety of the procedure and improvement of pain and quality of life for patients who received the lowest dose of ASCs (2×10^6 cells) (Pers *et al.*, submitted).

It might be intuitive to think that cartilage regeneration will be especially difficult to reach when the tissue is severely damaged[7]. The radiographic stage that would be optimal for MSC infusion is still not clearly defined although lesions of large size ($\geq$5.4 cm^2) have been associated with poor clinical and arthroscopic outcomes, suggesting a better benefit for patients with less severe OA[106]. Nevertheless, Orozco *et al.* did not report higher benefit with the four patients with early stage OA on the 12 patients enrolled, likely due to the small number of individuals[108]. All other studies included late stage OA patients[105,107]; Pers *et al.*, submitted. A summary of on-going or completed clinical trials on stem cell therapy in OA is given in Table I (ClinicalTrials.gov sources). All these data support the trophic action of MSCs for reducing synovial inflammation and protecting cartilage from degradation. Although the preliminary results from these studies seem encouraging for severe OA lesions, prospective studies should focus on OA patients with early radiographic stage in order to prevent or limit the structural progression of the disease. Further insight on the therapeutic utility of MSCs for OA patients will come from the on-going phase I and II trials.

Conclusion

OA is a complex disease characterized by the alteration of various molecular pathways in several compartments in the joint. Altered pathways are likely to be different depending on OA subsets (mechanically-induced OA, metabolic disorder associated OA, inflammatory OA, …), joint location (knee, ankle, hip, …) or individuals. In this context, cell therapy approaches using MSCs may be of high interest since they exert pleiotropic functions that may give therapeutic benefit on OA lesions. Preliminary results of preclinical and phase I or II clinical studies using BM- or adipose tissue-derived MSCs are promising since MSC therapy was shown to be safe and well-tolerated. Other approaches based on the use of embryonic stem cells (ES) or induced pluripotent stem cells (iPS) are currently under investigation for proposing therapeutic options or evaluating new drugs that could prevent cartilage degradation and modify the course of OA[109,110]. iPS can be generated from different tissues with significantly less invasive procedures than MSCs, reprogrammed towards the desired phenotype and used in regenerative medicine[111]. Together with the need of controlled long-term studies to confirm whether this new strategy of MSC-based therapy can improve pain and induce structural benefit,

Table I
Summary of clinical trials (on-going or completed) on stem cell therapy in OA (ClinicalTrials.gov sources)

Type of stem cells	Localization	Autologous or allogeneic	Phase study	ClinicalTrials.gov identifier	Nb patients enrolled	Status	Sponsor country
ASC	IA Knee	Autologous	I	NCT01585857	18	C	France
ASC	IA Knee	Autologous	I–II	NCT02219113	12	R	Russia
ASC	IA Knee	Autologous	I–II	NCT01300598	18	C	Korean
ASC + PRP	IA Knee	Autologous	I–II	NCT01739504	500	R	USA
BM–MSC	IA Knee	Allogeneic	I–II	NCT01586312	30	C	Spain
BM–MSC	IA Knee	Autologous	I–II	NCT01183728	12	C	Spain
BM–MSC	IA Knee	Autologous	II	NCT01459640	50	R	Malaysia
BM–MSC	IA Knee	Autologous	I–II	NCT02351011	12	R	Canada
BM–MSC	IA Knee	Autologous	I	NCT01207661	6	C	Iran
BM–MSC	IA Knee	Autologous	I–II	NCT01227694	15	C	Spain
BM–MSC	IA Knee	Autologous	I–II	NCT02123368	30	R	Spain
BM–MSC	IA Knee	Autologous	II	NCT01504464	40	C	Iran
BM–MSC	IA Knee	Autologous	I–II	NCT01183728	12	C	Spain
BM–MSC	IA Knee	Autologous	I–II	NCT01152125	10	R	India
BM–MSC	IA Knee	Autologous	I–II	NCT01485198	30	R	Mexico
BM–MSC	IA Hip	Autologous	I	NCT01499056	30	C	Iran
BM–MSC	IA Ankle	Autologous	I	NCT01436058	6	C	Iran
BM–MSC	IA Knee	Allogeneic	I	NCT01448434	72	R	Malaysia
UC–MSC	IV or IA Knee	Allogeneic	I–II	NCT02237846	40	R	Panama

ASC: adipose-derived stem cell; BM: bone marrow; UC: umbilical cord; PRP: platelet rich plasma; IA: intra-articular; IV: intra-venous; R: recruiting; C: completed study; Nb: number.

the possibility of using other stem cell-based approaches has to be evaluated.

Contributorship

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

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Conflict of interest

None.

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