Osteoarthritis and Cartilage 25 (2017) 737–741

Osteoarthritis and Cartilage

Brief Report

Stage-specific differences in secretory profile of mesenchymal stromal cells (MSCs) subjected to early- vs late-stage OA synovial fluid

A. Gómez-Aristizábal † ‡ [a b], A. Sharma ‡ § [a b], M.A. Bakooshli † # [c], M. Kapoor ‡ § [d], P.M. Gilbert † # †† [e], S. Viswanathan † ‡ § ‖ [**], R. Gandhi ‡ ¶ [*]

† *Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada*
‡ *The Arthritis Program, Toronto Western Hospital, Toronto, ON, Canada*
§ *Division of Genetics and Development, Krembil Research Institute, Toronto, ON, Canada*
‖ *Cell Therapy Program, University Health Network, Toronto, Canada*
¶ *Division of Orthopaedic Surgery, Toronto Western Hospital, University of Toronto, Toronto, ON, Canada*
# *Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, ON, Canada*
†† *Department of Biochemistry, University of Toronto, ON, Canada*

ARTICLE INFO

Article history:
Received 30 June 2016
Accepted 21 November 2016

Keywords:
Osteoarthritis
Mesenchymal stromal cells
Synovial fluid
Cytokines
CXCL8
Multiplex

SUMMARY

Objective: Although, mesenchymal stromal cells (MSCs) are being clinically investigated for their use in osteoarthritis (OA), it is unclear whether their postulated therapeutic properties are equally effective in the early- and late-stages of OA. In this study we investigated MSC cytokine secretion post-exposure to synovial fluid (SF), obtained from early- vs late-stage knee OA patients to justify a potential patient stratification strategy to maximize MSC-mediated treatment effects.

Method: Subjects were recruited and categorized into early- [Kellgren–Lawrence (KL) grade I/II, $n = 12$] and late-stage (KL-III/IV, $n = 12$) knee OA groups. SF samples were obtained, and their proteome was tested using multiplex assays, after 3-days culture, with and without MSCs. SFs cultured without MSCs were used as a baseline to identify MSC-secreted factors into SFs cultured with MSCs. Linear mixed-effect models and non-parametric tests were used to identify alterations in the MSC secretome during exposure to OA SF (3-days). MSCs cultured for 3-days in 0.5% fetal bovine serum (FBS)-supplemented medium were used to compare SF results with culture medium.

Results: Following exposure to OA SF, the MSC secretome contained proteins that are involved in tissue repair, angiogenesis, chemotaxis, matrix remodeling and the clotting process. However, chemokine (C-X-C motif) ligand-8 (CXCL8; chemoattractant), interleukin-6 (IL6) and chemokine (C-C motif) ligand 2 (CCL2) were elevated in the MSC-secretome in response to early- vs late-stage OA SF.

Conclusion: Early- vs late-stage OA SF samples elicit a differential MSC secretome response, arguing for stratification of OA patients to maximize MSC-mediated therapeutic effects.

Introduction

Osteoarthritis (OA) management is limited to either symptom alleviation or arthroplasty, leaving the underlying cartilage, bone and synovium pathology unaddressed. Recently, mesenchymal stromal cells (MSCs) have emerged as a promising cell-based therapy for OA[1] due to their immunomodulatory properties and multi-lineage differentiation capacity, including chondrogenic differentiation[2]. Intra-articular delivery of MSCs for OA treatment is being investigated in clinical trials, and safety, feasibility and preliminary efficacy of MSCs has been reported[1]. Questions remain about dose, carrier choice and whether MSCs are truly disease modifying.

* Address correspondence and reprint requests to: R. Gandhi, Toronto Western Hospital, 399 Bathurst St, Toronto, ON, M5T 2S8, Canada.
** Address correspondence and reprint requests to: S. Viswanathan, Krembil Discovery Tower, 60 Leonard Ave, 3KD-479, Toronto, ON, M5T 2S8, Canada.
E-mail addresses: sowmya.viswanathan@uhnresearch.ca (S. Viswanathan), rajiv.gandhi@uhn.ca (R. Gandhi).
[a] AGA and AS contributed equally to this work.
[b] Krembil Discovery Tower, 60 Leonard Ave, 5KD-415, Toronto, ON, M5T 2S8, Canada.
[c] Donnelly Centre for Cellular and Biomolecular Research, 160 College Street, Toronto, ON, M5S 3G9, Canada.
[d] Krembil Discovery Tower, 60 Leonard Ave, 5KD-413, Toronto, ON, M5T 2S8, Canada.
[e] Donnelly Centre for Cellular and Biomolecular Research, 160 College Street, Room 510, Toronto, ON, M5S 3E1, Canada.

http://dx.doi.org/10.1016/j.joca.2016.11.010

MSCs have shown both reparative and anti-inflammatory effects in animal models of OA, with contribution to cartilage regeneration, increased systemic levels of interleukin-10 (IL10), and reduction of joint-specific prostaglandin-E2[1]. However, these effects have not been fully confirmed in clinical trials. Importantly, the MSC secretome is considered important in inducing anti-inflammatory and anti-catabolic effects in tissues[3]. Identifying and quantifying the MSC secretome in response to different OA joint microenvironments are therefore critical steps towards elucidating the mechanism of action of MSCs in OA.

Importantly, it is unclear whether MSC trophic factor secretion is differentially modulated in the context of an OA joint; specifically, in early- vs late-stage OA. Since MSC trophic factor secretion can facilitate cartilage repair and reduce inflammation, interrogating the MSC secretome after exposure to different OA microenvironments is necessary to identify OA patient subsets that would most benefit from MSC therapy. Identifying and targeting those patients at the outset will result in unequivocal clinical outcomes in late-stage clinical trials; a lesson learned from subset successes of MSCs in GvHD (graft vs host disease), where MSCs were effective in gastrointestinal GvHD but not skin GvHD[4].

In this study, we systematically assess the MSC secretome after exposure to different synovial fluids (SFs) (cell depleted), from early- and late-stage OA patients, using multiplex immunoassays and report differential responses to OA SF stages.

Materials and methods

OA patients

To determine the impact of different OA microenvironments on the MSC secretome, subjects diagnosed with primary knee OA were studied. Grade I and II, defined by the Kellgren–Lawrence (KL) rating scale, were categorized as early-stage, while patients with KL-III/IV were categorized as late-stage. Samples were acquired from patients who provided written informed consent to participate in this study. The University Health Network Research Ethics Board approved the study and acquisition of human tissues (bone marrow acquired at Princess Margaret Hospital; Protocol ID: #06-446-CE, SF acquired at Toronto Western Hospital; #10-0455-AE). Patients were Caucasian, 30–65 years old and with 25–65 kg/m^2 body mass index (BMI). They were symptomatic, with enough severity for the need of intervention, and were off of any intra-articular corticosteroid for at least 3-months before SF draw. Patients with a diagnosis other than primary OA were excluded. Three donor-MSCs were used for downstream validation studies to verify secreted cytokines identified in the screen, performed with a single donor-MSC (Fig. 1).

Synovial fluid

SF was aspirated under sterile conditions, centrifuged 10 min at 2348 ×g, aliquoted and stored at −80°C (this ensured lysis of any remaining cells in the SF). SF samples were subjected to a single freeze–thaw cycle.

MSC isolation, culture, and selection

MSCs were acquired from bone marrow aspirated from the iliac crest of healthy donors, and expanded (up to passage 3–4) as previously described[5], using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). MSCs were cryopreserved and thawed >3 days before starting an experiment. MSCs were characterized for cell surface molecules (Supplementary Table 1) and trilineage differentiation. All MSCs exhibited immunomodulatory properties as assayed by T cell proliferation inhibition and induction of M2-Monocyte polarization (Supplementary Fig. 1).

Cytokine analysis

For multiplex analysis, MSCs were cultured for 3 days in 90% SF and 10% DMEM at 71.4 × 10^3 cells/ml. Briefly, MSCs were added to SFs by "injecting" MSCs, suspended in DMEM, into wells containing SF and later centrifuging to allow distribution of MSC suspension throughout the SF. For MSC-only (i.e. no SF) secretome analyses, cells were cultured in DMEM containing 0.5% FBS. Interleukin-6 (IL6), chemokine (C-X-C motif) ligand-8 (CXCL8), chemokine (C-C motif) ligand-2 (CCL2) and chemokine (C-X-C motif) ligand-12 (CXCL12) were selected for analyses as a preliminary multiplex (64 cytokines) screening of a single donor-MSC (Fig. 1 and Supplementary Table 3) identified IL6 and CCL2 as the two most highly secreted cytokines; CXCL8 and CXCL12 were selected as they were differentially secreted by MSCs in early- vs late-stage OA SFs. Analyses were performed on three donor-MSCs and 12 early- and late-stage OA patient SFs; each using a Bio-Plex MAGPIX 4-plex immunoassay (Bio-Rad, Mississauga, ON), as per manufacturer's instructions. SF viscosity was reduced before immunoassays by 1-h incubating samples with 2 mg/mL hyaluronidase (Sigma–Aldrich, Oakville, ON) at room temperature. Neat OA SF cultured for 72 h in the absence of cells (i.e., 'baseline') vs MSC treatment were used to determine the MSC levels of cytokines (ΔSF: pg/mL). Changes in cytokine levels were solely attributed to MSC secretion as no other cells were present.

Statistical analysis

Descriptive statistics were obtained. Samples were analyzed by OA stage [early- (n = 12) vs late-stage (n = 12) groups] after determining the net increase (SF_{+MSCs}–SF_{Neat}) in cytokines due to exposure to a single donor-MSC at the screening stage and to three independent donor-MSCs at the validation stage. Each donor-MSC was tested in duplicate with each SF and dependencies were removed by using the mean of duplicate measurements. Each SF treatment is considered independent, each coming from a different patient. Wilcoxon rank-sum test was used for comparisons between early- and late-stage OA SF groups for each donor-MSC. To account for the overall effect of early- and late-stage OA SFs on all donor-MSCs, we used linear mixed-effects models: with MSC and SF donors modeled as a random factors and SF stage as a fixed factor. P values less than 0.05 were considered to be statistically significant. OA stage effect sizes (r and marginal R^2, mR^2) were calculated as described in Supplementary Methods. All statistical analyses were performed with R version 3.1.2.

Additional methods

Additional methodology is described in Supplementary Methods.

Results

Screening

Since little is known on the differential protein secretion of MSCs in response to stage specific OA SFs (with only data on the effect of OA SFs on MSC gene expression[6–8]), we first screened the secretome of a single donor-MSC against a panel of 64 cytokines in response to early- (n = 12) and late-stage (n = 11) OA SFs (Supplementary Table 2). We confirmed that MSCs remain viable