Healthy chondrocyte-derived iMSCs exhibited enhanced chondroprotective, anti-inflammatory and analgesic effect than OA chondrocytes-derived iMSCs in a preclinical model of PTOA

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Introduction: Mesenchymal stem cells (MSCs) derived from various somatic sources including bone marrow and adipose tissue possess effective immunomodulatory properties and reduce inflammation that drive osteoarthritis (OA) progression. However, variability in MSC tissue source has resulted in inconsistent findings in preclinical and clinical settings. To overcome the problems associated with somatic MSCs, we propose the use of MSCs derived from induced pluripotent stem cells (iMSCs) that could represent a readily available, unlimited, homogenous cell source for OA therapy. We have previously shown that iMSCs derived from OA chondrocyte-iPSCs (OA-iMSC) suffer from disease memory that significantly reduced its chondrogenic potential as compared to iMSCs derived from healthy chondrocyte-iPSCs (AC-iMSC). In the present study, we aimed to establish whether memory of OA-associated chronic inflammation can impair the immunomodulatory capabilities of iPSCs and if such an impairment would inhibit treatment efficacy. We hypothesized that OA-iMSCs will continue to represent the features of their physiological origin and thus suffer from impaired immunomodulatory and anti-inflammatory capabilities, leading to inferior tissue preservation and analgesia in a preclinical model of post-traumatic (PT) OA.
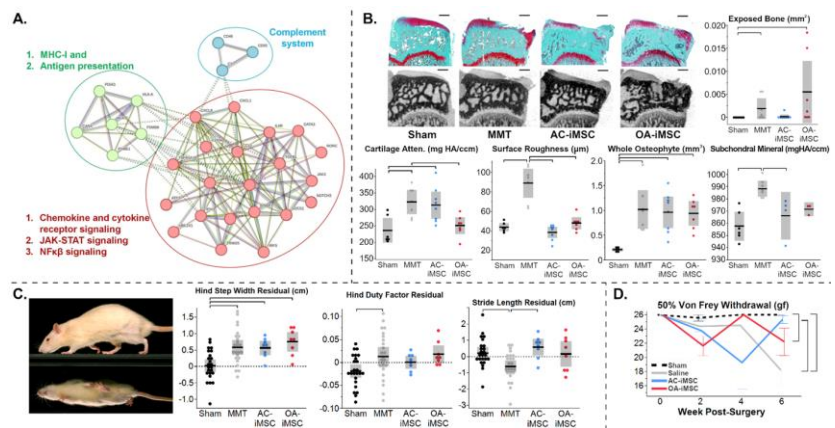
Methods: Lentiviral-based reprogramming was used for the generation of iPSCs from human chondrocytes isolated from healthy cartilage (AC-iPSCs) and osteoarthritic cartilage (OA-iPSCs). iPSCs were differentiated into mesenchymal progenitor intermediates (iMSCs) using our established direct plating method. Characterization of MSC-like features was performed using gene expression and immunophenotypic analyses. The pan-transcriptome profiling of these iMSCs was performed using bulk RNA-sequencing analysis for differentially expressed genes. Immunomodulatory effects of iMSCs were analyzed by their ability to modulate the expression and secretion of immunoregulatory factors upon stimulation with IL1β and IL1α (10 ng/ml, 24h). Single cell functional proteomics was analyzed using IsoCode microchip system using Human Adaptive Immune panel. Male Lewis rats (12-weeks old) were used for medial meniscal transection (MMT) surgery where both the medial collateral ligament (MCL) and the medial meniscus were transected to induce PTOA. AC-iMSCs, OA-iMSCs (1×10^6 cells in 50 μL HBSS), or saline were injected intra-articularly in MMT rats at 3 weeks post-surgery. Mechanical (secondary) allodynia and spontaneous gait was assessed longitudinally until the endpoint of 6 weeks. Tissue morphology was analyzed using contrast enhanced micro-computed tomography (EPIC-μCT) imaging and histology.

Results: Although AC-iMSCs and OA-iMSCs exhibited similar mesenchymal surface marker expression and comparable phenotypic features *in vitro*, pan-transcriptome analysis showed that AC-iMSCs exhibit distinct immune modulatory and inflammatory expression signatures that distinguishes them from OA-iMSCs. Transcriptome mapping for innate and adaptive immune regulation indicate that disease memory predisposes OA-iMSCs for impaired immunomodulatory potential as demonstrated by ineffective modulation of pro-inflammatory gene expression, whereas AC-iMSCs showed significant suppression of a battery of inflammatory genes. Moreover, functional clustering identified that AC-iMSCs exhibited enhanced immunomodulation by repressing three distinct clusters of inflammatory genes belonging to the Complement system, Antigen presentation, Chemokine/Cytokine receptor signaling involving NFκβ and JAK-STAT pathways (**Fig. 1A**). OA-iMSCs failed to modulate the expression of various inflammatory cytokines and chemokines such as *CCL5, CXCL3, CXCL8,* etc. after stimulation with IL1β and IL1α. However, AC-iMSCs showed significant suppression of inflammatory genes expression upon stimulation. Single cell functional proteomics further demonstrated that OA-iMSCs secrete higher levels of pro-inflammatory cytokines indicating that AC-iMSCs favor anti-inflammatory state upon pathogenic stimulation. To analyze the *in vivo* immunomodulatory effects of AC- vs OA-iMSCs, we evaluated the ability of these iMSCs to preserve joint health in a surgical model of PTOA. By 6 weeks, untreated MMT rats walked with a shuffle step (increased hind duty factor (p=0.032 vs sham), decreased stride length (p=0.009)) with wider hind steps (p<0.001) (**Fig. 1C**) and presented with allodynia (p=0.004) (**Fig. 1D**). OA-iMSCs did not resolve allodynia (p=0.361 vs saline) while rats treated with AC-iMSCs had no signs of allodynia (p=0.021). Both iMSC injections modestly improved gait to be similar to sham, though wider hind steps were still observed in both groups. OA-iMSCs decreased cartilage surface roughness (p<0.001 vs saline) and preserved proteoglycans (p=0.029), but still presented with severe damage including large lesions (p=0.711), and osteophytes (p=0.995), as well as elevated subchondral mineralization (p=0.169). AC-iMSCs had moderate benefits on tissue health, including decreased surface roughness (p<0.001), exposed bone (p=0.049) and subchondral mineralization (p=0.027), though proteoglycan loss (p=0.975) and osteophytes (p=0.977) remained prevalent (**Fig. 1B**).

Discussion: Our results showed that iMSCs derived from OA cartilage present with features of their physiological origin and exhibit impaired immunomodulatory potential compared to AC-iMSCs derived from healthy chondrocyte-iPSCs. Under a pathological environment, OA-iMSCs did not suppress inflammatory gene expression and cytokine section. However, AC-iMSCs showed enhanced immunomodulatory potential and greatly reduced the expression of major inflammatory pathway genes such as innate (MHC1, APC), adaptive immune, JAK-STAT and NFκβ signaling pathways. Additionally, single-cell responses of iMSCs demonstrated that under resting state OA-iMSCs were polarized to MSC1 phenotype to secret more inflammatory cytokines, whereas AC-iMSCs transformed into MSC2 phenotype to repress inflammation and thus have better potential to restore immunological homeostasis in degenerative joint disease. Consequently, AC-iMSCs performed better compared to OA-iMSCs in a preclinical model of PTOA with complete resolution of allodynic pain, improvement of functional gait, and increased potential for tissue preservation.

A. Interaction network showing distinct protein cluster modulated by AC-iMSCs upon inflammation stimulus; **B**. iMSCs moderately preserved joint tissue health, as measured by μCT. AC-iMSCs reduced exposed bone, cartilage surface roughness and subchondral bone mineralization. **C**. AC-iMSCs decreased the shuffle step phenotype but did not decrease hind step width. **D**. Untreated MMT rats had allodynic pain by 6 weeks. Only AC-iMSCs reduced this pain phenotype.

Significance: Our results demonstrated that disease memory in OA-iMSCs negatively influence their immunomodulatory potential and reduced their ability for cartilage preservation and analgesia in surgical induced OA model. Our results highlight the importance of erasing physiological memory of OA to prolong the healthy life of the articular chondrocytes and cartilage.