

differentiate into a chondrogenic lineage. Transforming Growth Factor- $\beta$  (TGF $\beta$ ) has a crucial role in chondrogenesis and can either signal via type I receptor ALK5 or ALK1 in chondrogenic cells. Downstream ALK5 and ALK1 lead to different intracellular signaling routes; Smad2/3 and Smad1/5/8 respectively. As TGF $\beta$  is required for induction of chondrogenic differentiation, we wondered whether TGF $\beta$  could by itself alter the receptor expression of ALK1 and ALK5, thereby modulating its own signaling pathway.

**Methods:** Human BMSCs and dedifferentiated ACs were chondrogenically (re)differentiated in pellet culture in serum-free chondrogenic medium containing 10nM dexamethasone and 25  $\mu$ g/mL ascorbic acid, with or without 10 ng/mL TGF $\beta$ 1. Pellets were harvested after 1, 4, 7, 14, 21, 28 and 35 days of chondrogenic induction for transcript analysis. Relative mRNA levels of ALK1, ALK5, collagen type II and aggrecan were measured using Q-RT-PCR. Values were corrected for ribosomal protein S27a. To further evaluate the quality of the extracellular matrix deposited by the cells, pellets were harvested after 21 and 35 days and stained with Safranin O/Fast Green (proteoglycans) or immunostained for collagen type II. An unpaired sample T-test was performed between TGF $\beta$ -stimulated and unstimulated pellets ( $n = 3$  pellets per time point).

**Results:** Only pellets stimulated with TGF $\beta$  displayed chondrogenesis as measured by gene expression of collagen type II and aggrecan, and immunohistochemical staining for proteoglycans and collagen type II. Contrary to unstimulated pellets that were devoid of cartilage-like tissue. In unstimulated BMSCs, ALK1 expression was upregulated during the first 4 days of culturing, while this upregulation was absent in TGF $\beta$ -stimulated pellets. As a result, ALK1 expression remained stably lower ( $\sim 4$ -fold;  $p < 0.01$ ) in the TGF $\beta$ -stimulated compared to the unstimulated condition from day 4 until the end of the culture period. In contrast, ALK5 expression was continuously increased by TGF $\beta$  within the first 14 days of induction, remaining constantly higher ( $\sim 10$ -fold;  $p < 0.001$ ) than in unstimulated pellets during the remainder of the culture. In pellets formed with dedifferentiated ACs, ALK1 expression rapidly decreased in TGF $\beta$ -stimulated pellets and stayed at a lower ( $\sim 3$ -fold;  $p < 0.01$ ) level than in the unstimulated pellets until day 35. Expression of ALK5 was higher ( $\sim 10$ -fold;  $p < 0.0001$ ) in ACs stimulated with TGF $\beta$  than in the control condition on day 1. After day 1, ALK5 expression remained higher ( $\sim 2$ -fold;  $p < 0.05$ ) in TGF $\beta$ -stimulated pellets compared to unstimulated pellets, although the difference became smaller with time during AC redifferentiation.

**Conclusions:** Our data show that TGF $\beta$  modulates ALK1 and ALK5 expression in chondrogenic cells by stimulating ALK5 expression whilst dampening ALK1 expression. This shows that TGF $\beta$  not only directly induces chondrogenic differentiation, but that it also modulates the signaling pathways itself by altering the expression of the TGF $\beta$  type I receptors, ALK5 and ALK1 favoring ALK5. Unraveling the role and regulation of these receptors will help us to further improve the formation of stable cartilage by BMSCs.

## 799

### EARLY INTRA-ARTICULAR INJECTION OF MESENCHYMAL STEM CELLS PREVENTS SYNOVIAL INFLAMMATION AFTER MEDIAL MENISCAL RELEASE IN A RABBIT MODEL OF OSTEOARTHRITIS

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**Purpose:** Osteoarthritis (OA) is characterized by a catabolic and inflammatory joint environment. One of the main objective in the management of OA is to dampen local inflammation in an attempt to slow down cartilage degradation process and to relieve pain. Until recently, Mesenchymal Stromal Cell (MSC) therapies for OA treatment have mainly relied on their chondrogenic properties for cartilage repair. The anti-inflammatory and immunomodulatory properties of Umbilical Cord-Derived MSC (UC-MSC) has been rarely studied in the context of the onset of the disease.

In the present study, we investigated the anti-inflammatory and anti-catabolic effects of UC-MSC in a rabbit model of first stage mild OA. We examined the differential ability of an early or delayed intra-articular (IA) injection of UC-MSC in preventing synovial inflammation, one of the first events leading ultimately to cartilage damage. The interactions between MSC and the synovial membrane with regards to MSC anti-inflammatory potentials were evaluated especially by analyses of the expression of genes related to inflammation and matrix turnover. The

nature of the effects of UC-MSC on stimulated synoviocytes were further investigated in vitro.

**Methods:** Medial meniscal release (MMR) was performed in rabbit knee joints (day 0). A single early or delayed intra-articular (IA) injection of UC-MSC was realized (day 3 or 15). At day 56, gross morphology, Equilibrium Partitioning of an Ionic Contrast agent via micro-CT (EPIC- $\mu$ CT) and histology were performed to grade OA lesions. Gene expression of inflammatory cytokines and metalloproteases was measured, in synovial tissue harvested from the OA knees. In order to study the paracrine effects of UC-MSC, control rabbit primary synoviocytes were incubated with UC-MSC-conditioned medium or control medium under pro-inflammatory stimulus for 48 h (IL-1 $\beta$  (10ng/ml)).

**Results:** As expected, at day 56, MMR procedure leads to changes consistent with early OA in the medial compartment of the rabbit knee joints. Cartilage fibrillation, inflammatory cytokines and metalloproteases gene expressions were lower in the early IA group than in the delayed group. OA synovium gene expression analysis showed a reduction in the expression of both the inflammatory cytokines (IL1 $\beta$ , TNF $\alpha$ ) and the metalloproteases (MMP-1, -3, -13) in the early-injected group compared to the MMR group. UC-conditioned medium exerted anti-inflammatory and anti-catabolic effects synoviocytes stimulated with IL-1 $\beta$ . These results confirmed UC-MSC possess paracrine activity that target stimulated synoviocytes.

**Conclusions:** This study shows the efficiency of a single early IA injection in preventing OA signs in rabbit knee following MMR. Early UC-MSC IA injection might be more efficient to reduce inflammation and prevents OA progression than delayed UC-MSC IA injection.

## 800

### MAPPING OF THE WNT PATHWAY IN OSTEOARTHRITIC AND HEALTHY SUBJECTS REVEALS AN INHERENTLY ALTERED PATTERN IN OSTEOARTHRITIS MESENCHYMAL STEM CELLS

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**Purpose:** Osteoarthritis (OA) is characterized by homeostatic alterations, resulting in abnormal restoration of damaged joint tissues by progenitor mesenchymal stem cells (MSCs). Role of WNT signaling is crucial in this process regulating MSCs proliferation and differentiation to the osteogenic and chondrogenic lineages, the main structural cell types of the joint. Using a targeted gene array, we aimed to describe the expression fingerprint of this pathway in OA pathogenesis mapping the Wnt pathway integrity in MSCs and mature chondrocytes.

**Methods:** Total RNA from MSCs and mature chondrocytes (C) cell cultures was isolated from 10 OA patients (OA-MSCs = 3 and OA-C = 7) and 11 healthy controls (MSCs = 8 and C = 3). Analysis of differential transcript expression of two metalloproteases, two collagen genes and 20 genes mapping the WNT signal transduction pathway were evaluated using real-time quantitative PCR.

**Results:** Compared with healthy MSCs, expression of the transcription factor gene *LEF1* and the *WNT5A* ligand were significantly down-regulated and *ADAMTS4* and *BMP2* were upregulated in OA-MSCs. Healthy mature chondrocytes showed an upregulation of *BMP2*, *SOX9* and *JUN*. Downregulation of the *JUN* transcription factor concomitant with an upregulation of kinases *CSNK1A1* and *GSK3B* was present in OA-Chondrocytes compared with healthy chondrocytes that also showed an upregulation of *LRP5* coreceptor compared with OA-MSCs.

**Conclusions:** Expression of WNT related genes show a pattern of variation in expression among MSCs and chondrocytes from OA and healthy samples that point to inherent alterations in OA-MSCs that determine their future chondrogenic phenotype in terms of a more reduced chondrogenic potential and an increased catabolic environment.

## 801

### SYNOVIAL ACTIVATION DRIVES ANTI-INFLAMMATORY EFFECTS OF ADIPOSE-DERIVED STEM CELLS AFTER LOCAL ADMINISTRATION IN EXPERIMENTAL OA WHICH IS REFLECTED BY S100A8/A9 LEVELS IN THE SERUM

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