Purpose: The transcription factor Sox9 plays an important role in the regulation of skeletal growth and cartilage formation. De novo mutations in the coding region of Sox9 cause campomelic dysplasia, an autosomal dominant, frequently lethal disease. Moreover, Sox9 is known to control the initial steps of chondrogenic differentiation of progenitor cells. However, this chondrogenesis needs to take place in a diseased joint with an altered environment. In this environment inflammatory mediators will be present in elevated levels and these will activate inflammatory pathways in differentiating chondrocyte precursors. We examined the role of two inflammatory pathways in chondrocyte differentiation, the NF-kB and SOCS-3 pathway as possible therapeutic targets to optimize cartilage regeneration.

Methods: Human mesenchymal stem cells (hMSC) (Lonza) were differentiated into the chondrogenic lineage. Cells were pelleted in serum free chondrogenic medium supplemented with TGF-beta and BMP-2. Interleukin-1 (IL-1) and osteoarthritic synovium derived conditioned medium, both a model for inflammatory stimulation, were added to hMSC undergoing chondrogenic differentiation.

Two downstream pathways known to be induced by catabolic factors such as IL-1 were examined: the NF-kB and the SOCS-3 pathway. To study the specific role of the NF-kB pathway cells were lentivirally transfected with P65 siRNA hairpin to block P65 expression. Activation of the SOCS-3 pathway was mimicked by lentiviral overexpression of SOCS-3. Chondrogenesis was examined by determining mRNA levels of chondrogenic markers COL2A1 (collagen type II) and ACAN (aggrecan). Proteoglycan deposition was analyzed by safranin O staining of histological sections.

Results: Chondrogenic differentiation, as measured by expression of type II collagen and aggrecan and safranin O proteoglycan staining, was induced in the hMSC already strongly at two weeks under the described anabolic conditions. IL-1 as well as osteoarthritis synovium-conditioned medium inhibited chondrogenesis of hMSC. Conditioned medium induced SOCS-3 mRNA strongly in the hMSC undergoing chondrogenesis. IL-1 also induced SOCS-3 mRNA, albeit at a lower level. Lentiviral overexpression of SOCS-3 lead to total inhibition of chondrogenesis already in the condition without catabolic factors. In addition, blocking the NF-kB pathway P65 siRNA resulted in complete inhibition of chondrogenesis, even under anabolic conditions. Indicating that NF-kB signaling is required for normal chondrogenesis

Conclusions: Our study shows that the NF-kB pathway appears to be indispensable for chondrogenesis. This demonstrates NF-kB is unsuitable as a target to stimulate chondrogenesis in an inflammatory milieu. SOCS-3 was induced by catabolic factors and overexpression of SOCS-3 potently inhibited chondrogenesis. SOCS-3 is therefore a potential candidate target to modulate the inhibiting effect of catabolic factors on chondrogenesis and blocking SOCS-3 in diseased joints could improve cartilage repair through de-repression of chondrogenesis.
knockdown seems to impair cell growth and alters cell cycle distribution. An experimental set up, using a RT-PCR based cell cycle pathway array showed a reproducible up regulation of LOC688900 in rMSC, which encodes Schlafen-1, a protein known as an inducer of cell cycle arrest in T-lymphocytes and murine fibroblasts.

Conclusions: Based on these knockdown studies we hold a promising tool for further in vitro analyses and differentiation studies in MSC. According to the present data, we conclude an involvement of Sox9 in cell cycle progression and thus cell proliferation, and suggest an up regulation of LOC688900 as a consequence of Sox9 down regulation.

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PERLECAN/HYALURONAN-BASED BIOMATRICES PROLONG THE DELIVERY OF CARTILAGE-INDUCING GROWTH FACTORS: A PROMISING SYSTEM FOR STIMULATING SELF-REPAIR OF DAMAGED ARTICULAR SURFACES

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Purpose: During the early stages of osteoarthritis (OA), growth factors with high affinity for both cartilage and bone extracellular matrix are released and become involved in the repair of lesions. Recently, bone morphogenetic protein 2 (BMP-2), a growth factor with heparan sulfate (HS) binding capability, demonstrated anabolic cartilage activity when locally administered in the joint space of OA animal models. Because clearance of small compounds from the synovial fluid into the lymphatic system is efficient, the purpose of this study was to characterize the mixed synovial cell population (MSCP) and purified type-B synovial fibroblasts (SFB) derived from pig synovium by using various known cell-surface markers and evaluate their chondrogenic differentiation potentials using a chondrogenic media containing growth factor TGF-β1.

Methods: Cells were isolated from synovium of porcine knee joints. SF-Bs were negatively isolated by magnetic bead separation using Dynal CD14.

Flow cytometry analysis was performed with MSCP or SFB cells. Direct conjugated Ab used for flow cytometry included anti-CD44-fluorescein isothiocyanate (FITC), CD90-FITC, CD45-FITC, CD34-FITC, CD11b-phycocerythrin (PE). Unconjugated Ab is anti-CD14 and appropriate secondary Ab. Analysis was performed with FlowJo software. 0.5×10⁶ cells were centrifuged at 1000 rpm for 10 minutes. With the supplement of TGF-β1, pellets were cultured in defined differentiation medium, at 37°C; 5% CO₂. Pellets were harvested for biochemical and histological analyses, and digested (2 hr at 60°C) in 300 μl papain (125 μg/ml papain, 5 mM cysteine). DNA content was measured via fluorometric assay using Hoechst 33258. GAG concentration was measured by the dimethyl-methylene blue dye assay.

Figure 1. Surface molecule profile of the mixed synovial cell population (MSCP) and purified type-B synovial fibroblasts (SFB) using flow cytometry.

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CHARACTERIZATION AND DIFFERENTIATION OF PORCINE SYNOVIIUM-DERIVED MSCS

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Purpose: Articular cartilage damage has a very low spontaneous healing capacity. A cell-based therapy with an appropriate source would offer the opportunity to promote articular cartilage repair. Stem cells are characterized by their multipotentiality and capacity for self-renewal and may represent units of active regeneration for tissues damaged as a result of trauma or disease. Synovium can be easily harvested. Many attempts have been made to develop a cell-surface antigen profile for the better purification and identification of MSCs. However, the identification of MSC subpopulations in culture has been hampered by the lack of appropriate markers. Thus there is a clear need to develop strategies leading to the identification of potential MSC (sub)population markers. The objective of this study was to characterize the mixed synovial cell population (MSCP) and purified type-B synovial fibroblasts (SFB) derived from pig synovium by using various known cell-surface markers and evaluate their chondrogenic differentiation potentials using a chondrogenic media containing growth factor TGF-β1.

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Pellets were fixed in 1:1 methanol/aceton solution at 4°C, embedded in paraffin, sectioned at 5 μm, and stained with Weigert’s hemotoxylin and Safranin-O/Fast Green for sulfated GAGs and immunostained for type II collagen. Relative mRNA levels of collagen I, II and Aggrecan were determined by qPCR.

Statistics were performed using one-way ANOVA for at least n=4 where p<0.05 was considered significant.

Results: To characterize the mixed synovial cell population and purified type-B synovial fibroblasts flow cytometry was performed. The positive markers in MSCP were CD14, CD44, CD90, cadherin-11. After purification, the macrophage marker CD14 disappeared