under Good Laboratory Practise (GLP) conditions, after local intra-articular (IA) injection in mice.
Methods: Clinical grade ASC were isolated from subcutaneous abdominal fat, expanded in α-MEM medium with 10% platelet lysate and used at passage 1. ASC were injected either IA (106 cells/knee joint) or systemically (IV) via the tail vein (106 cells) of SCID mice. At different time points (day 11, 28, 90 186), 10 mice were euthanasized and several organs/tissues recovered. After DNA extraction, qPCR was performed using primers specific for human Alu or murine actine sequences.
Results: Quantification of hASC engraftment was performed through detection of human-specific Alu sequences and normalization using murine actine sequences on DNA extracted from 14 different organs or tissues. Using serial ten-fold dilutions of hASCs in murine MSCs, a linear correlation curve between the number of ASC and Alu signal was established. This curve was comparable between different cell samples and the detection limit was 0.005% hASCs. After IA injection, hASCs were detected in 10/10 mice at the different time points. They were in the joint in more than 90% of mice for the first 3 months and were still observed in 60% of the mice after 6 months. At day 11 and 28, approximately 15% of the injected cells were recovered in various organs. Cells were predominantly observed in the knee joints, bone marrow and fat. After 3 and 6 months, 1.5-4% of infused hASC were still detected in the joint, bone marrow, fat and muscle. After systemic injection, hASC were detected in 5 and 4/12 mice in one or two organs at day 11 and 28, respectively. Only 1.4 and 0.8% of the total infused cells were detected predominantly in lung, intestine, stomach, liver and brain.
Conclusions: Contrary to a systemic route, the IA injection of hASC allowed their survival on the long-term. Cells were predominantly localized at the site of injection but also, in the stem cell reservoirs, bone marrow and fat tissue.

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INHIBITORY EFFECT OF MIR-29A ON THE CHONDROGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Purpose: Mesenchymal stem or stromal cells (MSC) are multipotent cells that can differentiate into different lineages, particularly osteoblasts and chondrocytes. The differentiation process of MSC is regulated by various molecules among which Sox9 and Runx2 are key transcription factors leading, respectively to chondrogenesis or osteogenesis. Recently, a new class of regulating factors, namely microRNAs (miRNAs), has been shown to be important for differentiation processes but few miRNAs have been shown to regulate chondrogenesis. The objective of this study is therefore to identify miRNAs involved in the chondrogenic differentiation of MSC.
Methods: MiRNA arrays have been done using RNA samples of MSC (day 0) and MSC-derived pre-chondrocytes (day 3). Analysis of miRNAs, their putative targets and of transcription factors putatively binding to their promoter regions was performed using several prediction softwares, in particular Targetscan. Pre-miRs and antagomiRs were transfected in MSC twice (day -4 and -1) using oligofectamine and chondrogenic differentiation was induced by culture of MSC in micropellets in inductive medium for 21 days. Expression of chondrocyte markers was performed by RT-qPCR.
Results: Analysis of results from the miRNA arrays together with those from DNA arrays already available in our laboratory indicated that a little number of transcription factors was theoretically able to regulate the majority of the miRNAs that were modulated at day 3 of chondrogenesis. Among the transcription factors, Sox9 and YY1 can putatively bind to the promoter region of miR-29. Using real-time RT-PCR, we observed that the expression level of miR-29 progressively and highly decreases during the chondrogenic differentiation. Transfection of Sox9 or YY1 in the Stro-1A MSC cell line significantly reduced the expression of miR-29, while transfection of both factors totally abolished its expression. The effect of gain- and loss-of-function of miR-29 during the chondrogenic differentiation of MSCs by transfecting pre-miRs or antago-miRs confirmed the role of miR-29 during chondrogenesis.
Conclusions: Our preliminary data show that, during chondrogenesis, miR-29 expression is down-regulated, probably through the interaction of Sox9 and YY1 on the miR promoter region. Because miR-29 has been described to regulate different targets (DKK1, sFRP2, Kremen2 and CDK6 whose expression decreases during differentiation), future experiments will investigate whether these target genes are modulated by miR-29.

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MIGRATION POTENTIAL OF MESENCHYMAL STEM CELLS DERIVED FROM OSTEOARTHRITIC PATIENTS AND THEIR SUITABILITY FOR IN SITU TISSUE REPAIR

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Purpose: In situ tissue engineering based on factor-guided recruitment of mesenchymal stem cells (MSC) is suggested as promising approach for the treatment of joint cartilage and underlying bone lesions of patients with osteoarthritis (OA). First strategies to treat focal cartilage defects have reached clinical level, but focusing on OA, fundamental studies on feasibility are necessary. The aim of our study was to compare the chemokine-dependent migration potential and molecular changes during migration of MSC derived from OA patients and normal donors.
Methods: MSC were isolated from femoral heads derived from end-stage OA patients. The chemokine receptor profile of culture expanded MSC was analyzed on the gene expression level (PCR, Affymetrix microarray) and by immunohistochemistry. A 96-well plate chemotaxis assay was used to investigate the migratory response to potent chemokines known from normal donor MSC. The chemokines leading to the most pronounced migration response were further investigated. The intra- and extracellular expression of chemokine receptors of these chemokines was quantified by FACS analysis. Transcriptomic response to chemokine stimulation of OA MSC was analyzed on the global molecular level by microarray analysis. The results were compared with normal donor data.
Results: On the gene expression level, all OA MSC presented the chemokine receptors CCR1, CCR3, CCR5-11, XCR1-6 and CXCR1. Immunohistochemistry confirmed the results for CCR1, CCR6, CCR7, CCR9 and CXCR2-6, Donor-dependent results were obtained for CCR5 and CXCR1. MSC from OA donors showed a dose-dependent migration towards chemokines CCL2 (MCP1), CCL25 (TECK), CXCL12 (SDF1-alpha) and CXCL8 (IL8). The strongest migration was measured after the use of TECK in a concentration of 1000 nM. The corresponding receptor CCR9 was detected intra- and extracellularly by flow cytometry. During exposition with TECK, alterations on gene expression were detected involving genes of pathways related to homing of bone marrow cells (CXCL2, -3, PDE4B), cytoskeletal and membrane reorganization (CXCL8, IGFBP1) and cell movement (CXCL6, PTGS2, TGM2).
Conclusions: MSC from OA donors present distinct chemokine receptors and migrate towards potent chemokines. This is consistent with available data of normal donor MSC. Gene expression analysis indicated the activation of pathways involved in the initiation of chemotaxis and following migration. The Results proof, that MSC from OA patients fulfil the basic requirements for in situ tissue engineering. In accordance with experiences from normal donor MSC, TECK may be also a promising factor for the in situ recruitment of MSC from OA patients.

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AN IN VIVO TRACKING SYSTEM TO INVESTIGATE THE MIGRATION OF BONE MARROW STEM CELLS IN AN OSTEOCHONDRAL DEFECT MODEL

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Purpose: To develop an in vivo system to monitor the migration of bone marrow-derived MSCs (BMSCs) in an osteochondral defect model.
Methods: Chemotaxis assays were performed in a Boyden chamber to determine the most effective chemotactic factor. For in vivo study, BMSCs were labeled with fluorescent nanoparticles and injected into the marrow cavity of nude rats through osteochondral defects created in the distal femur. The defect was sealed with HCF (heparin-conjugated fibrin) or PDGF (platelet-derived growth factor)-AA-loaded HCF to induce the migration of BMSCs. The in vivo fluorescence was monitored for 21 days and the osteochondral defect was evaluated macroscopically and histologically after the period.
Results: The in vitro studies confirmed that PDGF-AA had the most potent chemotactic effect of the factors tested, and possessed the greatest number of receptors in BMSCs. In the HCF-only group, the