possibility of long-term immunological rejection of the allograft. In the future, it will be necessary to carry out these studies using an autograft model.

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REPEATED SUB-THRESHOLD DOSING OF TGF-BETA1 IS INEFFECTIVE IN STIMULATING HUMAN BONE MARROW CELLS: IMPLICATIONS FOR CARTILAGE REPAIR STRATEGIES

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Purpose: Articular cartilage injury and degeneration are leading causes of disability. Since the articular cartilage has a limited capability to heal itself, improving cartilage repair is an important strategy to reducing pain and disability. Transforming growth factor-beta 1 (TGF-β1) induces chondrogenesis of human bone marrow cells (hBMC), an important cell source for cartilage repair. A challenge to in vivo administration of TGF-β1 is understanding the dosing regimens that would effectively stimulate hBMC and improve cartilage repair. The aims of this study are to test the hypothesis that (1) TGF-β1 signaling can be induced and maintained by either single or repeated administrations in vitro, and (2) hBMC mediated cartilage repair cells remain responsive to sustained exposure to TGF-β1 in vivo.

Methods: To test hypothesis 1, hBMC were stimulated with single administration of 5, 10 or 15ng/mL of TGF-β1 and the expression of TβRII and phospho-SMAD3 compared with multiple administrations of TGF-β1 by Western Blot. Protein was isolated at 0, 2h, 4h, 1d, 3d and 7d and after the last administration of TGF-β1. To test hypothesis 2, TβRII expression were assessed in athymic rat osteochondral defects at 4 weeks following implantation of hBMC transduced with AAV-GFP or AAV-TGF-β1 by immunohistochemical staining.

Results: In vitro, TβRII expression increased significantly at 2h post-stimulation with single administration of 15ng/mL of TGF-β1, and at 4h and 1d post-stimulation with single administration of 10ng/mL of TGF-β1. Phospho-SMAD3 expression increased at 2h post-stimulation with single administration of 10ng/mL of TGF-β1. However, the expression of both proteins declined thereafter. In contrast, multiple administrations of 10ng/mL of TGF-β1 led to increases in TβRII and phospho-SMAD3 expression for up to 7 days post-stimulation.

In vivo, TβRII was strongly expressed throughout the repair tissues of osteochondral defects receiving hBMC expressing TGF-β1, but there was weak and sparse expression of TβRII in repair tissues of osteochondral defects receiving hBMC expressing GFP.

Conclusions: The data show that the effects of single administration of TGF-β1 in vitro are transient suggesting that repetitive administrations of 10ng/mL of TGF-β1 may be required to sustain cellular responsiveness in vivo. In vivo, cartilage repair cells remain responsive to repeated exposure to TGF-β1. The implication for in vivo repair strategies are that sustained exposure of hBMC to TGF-β1 is critical and can be achieved using AAV-vectors.

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ANTAGONIZING ENDIGENOUSLY EXPRESSED BONE MORPHOGENETIC PROTEINS IMPAIRS CARTILAGE EXTRACELLULAR MATRIX REPLENISHMENT IN VITRO

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Purpose: To investigate the role of bone morphogenetic proteins (BMP) in intrinsic cartilage repair following tissue damage.

Methods: Cartilage explants were obtained from metacarpophalangeal joints of adult steers. Cartilage damage was induced by depleting extracellular matrix by trypsin digestion. Samples were maintained in serum-free basal medium (BM) with and without the addition of either Noggin, Noggin + insulin-like growth factor (IGF)-1 and Noggin + Fetal calf serum (FCS). Undigested and unstimulated explants served as negative controls. At days 7, 14, 21, 28 and 35 biosynthesis of matrix macromolecules was assessed by [35S]Sulfate incorporation. Additionally, histological analysis using toluidine blue staining and quantitative real-time PCR on distinct BMPs was performed.

Results: Endogenous expression of BMPs was upregulated following cartilage damage; simultaneously, matrix macromolecule synthesis was increased. Blocking of BMP activity using the BMP-antagonist Noggin resulted in a significant reduction of glucosaminoglycan neo-synthesis. While FCS overcame the anti-anabolic effects of Noggin, the addition of IGF-1 had no significant stimulatory effect on matrix macromolecule production.

Conclusions: We conclude that the increased expression of BMPs after cartilage damage together with a BMP-dependent increase in matrix macromolecule synthesis indicates a functional role of these growth factors in intrinsic cartilage repair.

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AUTOLOGOUS MATRIX INDUCED CHONDROGENESIS (AMIC PLUS) FOR THE TREATMENT OF PATELLAR CARTILAGE DEFECTS IN THE KNEE

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Purpose: The present study was designed to evaluate the AMIC plus technique for the treatment of symptomatic patellar cartilage defects in the knee. MRI was used for the morphological analysis of cartilage repair.

Methods: The AMIC plus technique (combination of the original AMIC technique and the use of platelet rich plasma) was used for the treatment of symptomatic chondral and osteochondral patellar lesions in the knee. Five patients were clinically prospectively evaluated with use of the Knee Injury and Osteoarthritis Outcome Score (KOOS), a Visual Analogue Scale (VAS) for pain, the Tegner activity level scale and Kujala scale preoperatively and at 12 and 24 months of follow-up. All 5 patients had consented to follow the postoperative MRI evaluation protocol. MRI data were analyzed based on the original MOCART (Magnetic Resonance Observation of Cartilage Repair Tissue) scoring system.

Results: A statistically significant clinical improvement became apparent after 24 months of follow-up. The MOCART scoring system revealed no significant deterioration or improvement of the repair tissue between one and two years of follow-up. Twenty-four months after the operation hypertrophy was found in 40%. Subchondral bone changes and intralesional osteophytes were seen in all cases (100%). Synovitis and adhesions were not observed in the study patients at that time of follow-up.

Discussion: AMIC plus resulted in clinically significant improvement in all patients. The favourable clinical outcome of the AMIC plus technique was not confirmed by the MRI findings as determined by the MOCART score. More specifically, all cases showed subchondral lamina and bone changes, including intralesional osteophytes, were observed.

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HIGH-THROUGHPUT TRANSCRIPTION PROFILING REVEALS CANDIDATE GENES INVOLVED IN REGENERATION AFTER EXPERIMENTAL JOINT DAMAGE IN THE NEWT

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Purpose: In contrast to mammal joint injuries that cannot be treated causatively so far, amphibians are able to regenerate whole limbs and can endogenously cure local articular tissue lesions. Therefore, we aimed to decipher regeneration-relevant molecular factors by analyzing the differential gene expression profile of damaged vs. intact knee joints of adult newts using cDNA array technology.

Methods: Knee joint damages of adult newts (Notophthalmus viridescens) were induced by surgical removal of the femoral cartilage. After total RNA isolation from whole knee joints at different time points after damage hybridization onto cDNA microarrays was performed, and selected differentially expressed genes were being validated by PCR techniques.

Results: Based on the array spots usable for analysis after hybridization, we filtered those genes that were at least 2-fold differentially expressed in at least 3 of 4 replicates in the same direction. Accordingly, 26, 22 and 3 as well as 4, 11 and 9 known genes and many unknown transcripts were found to be up- or downregulated at days 10, 20 and 40, respectively. Amongst known genes, there were matrix components (decorin, biglycan, fibronectin, collagens, tenascin), anabolic and catabolic factors (e. g. TIMP, MMPs, cathepsins) as well as different signalling molecules. First PCR validations confirmed the gene expression differences between damaged and intact joints.