sheets that can be created pose problems in the clinical setting. To alleviate such issues, we have been investigating the use of allogeneic chondrocyte sheets for knee cartilage repair. In another ongoing clinical study, chondrocytes obtained from surgery of young polydactyly patients are being stocked and used, as necessary for transplantation, to create chondrocyte sheets. The polydactylly chondrocyte sheets will then be transplanted to patients, and the safety and effectiveness of treatment are to be evaluated as endpoints in this study.

To establish chondrocytes derived from polydactyly patients as a source for cell sheets, we have previously verified their safety by performing subcutaneous transplantation to severely immunodeficient mice (NOG mice) to test for tumorigenicity, array CGH, and G band for karyotype analysis. To further evaluate the properties of polydactylly chondrocytes in this study, we conducted a comparative microarray analysis of chondrocytes obtained from young polydactyly patients and those obtained from adult knees. We report genes unique to polydactyly chondrocytes, and several miRNA candidates that may be responsible for modulating their gene expression.

**Methods:** Experiments were performed under the approval and guidance of the Clinical Research Review Committee of Tokai University School of Medicine. Informed consents were obtained in all cases. Cartilaginous tissue from young polydactyly patients (6 patients; age, 11-16 months; average age, 13 months) was collected, minced, and digested in collagenase, and the isolated cells were cultured and treated as polydactylly chondrocytes. Cartilaginous tissue from non-loading parts of knees of adult patients with anterior cruciate ligament (ACL) injury (3 patients; age, 15-31 years; average age, 22.3 years) was obtained, and adult knee chondrocytes were similarly isolated and cultured. Total RNA was extracted from both cultured chondrocytes, and Agilent Human GE 4x44K v2 and Agilent Human miRNA Microarray (V3) 8x15k were utilized to map gene and miRNA expressions, respectively. Agilent GeneSpring GX version 11.0.2 was used to identify and analyze genes and miRNAs with changes in expression of greater than 1.5 fold (student t-test P-value < 0.05).

**Results:** Microarray data from polydactylly chondrocytes (n=6) and adult knee chondrocytes (n=3) were obtained and analysis was performed on probes showing significant signals in 2 or more samples. We identified differences in gene expression between polydactylly chondrocytes and adult knee chondrocytes in 2,323 out of 23,678 probes. Out of the 2,323 probes, 1,304 had higher expression and 1,019 probes had lower expression in polydactylly chondrocytes. Furthermore, of the 140 probes for miRNA expression, 8 probes had higher expression and 22 had lower expression in polydactylly chondrocytes. Pathway analysis identified no less than 3 miRNA candidates that may regulate gene expression of key genes in polydactylly chondrocytes compared with adult knee chondrocytes.

**Conclusions:** We were able to identify several miRNA candidates that characterize the difference between polydactylly chondrocytes and adult knee chondrocytes. Through further studies, we may be able to use synthetic nucleic acids that mimic miRNA activity to give young polydactylly chondrocytes adult-like characteristics.

603 CARTILAGE REGENERATION AND INTERMITTENT HYDROSTATIC PRESSURE: A ROLE FOR MSCs

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**Purpose:** Cartilage regeneration is observed after joint distraction in the treatment of severe knee osteoarthritis (Wiegant et al 2013). More than 20%–denuded bone is completely filled in 1 year, which cannot be solely the result of matrix synthesis by resident chondrocytes. We hypothesize that MSCs play a trophic role on resident chondrocytes. The intra-articular intermittent hydrostatic pressure (IHP) in the human knee during distraction is thought to induce activation of this trophic effect. The current study evaluates the effect of IHP on in vitro expression of regenerative mediators in hMSCs.

**Methods:** The IHP culture setup consists of two vessels, a pressure vessel and a control vessel. IHP between Patm and Patm – 15 kPa, (as found in vivo) is applied to the cells at a frequency of 0.33Hz. The vessels are placed in a regular incubator (T = 37 °C, [O2] = 20%, [CO2] = 5%, RH = 95%). Bone marrow derived human MSCs (n=5 donors) monolayer culture was performed in the IHP setup for 6h, 24h and 48h. mRNA expression levels for a set of cartilage-homeostasis related genes were determined, using hypoxanthine phosphoribosyltransferase (HPRT1) as a reference gene. Targets were: fibroblast growth factor 1, 2 and 18 (FGF1, 2, 18), tissue inhibitor of metalloproteinase 1 and 2 (TIMP1,2), transforming growth factor β 1 (TGFβ1), insulin-like growth factor 1 (IGF1) and hepatocyte growth factor (HGF). The comparative Ct method (∆∆Ct) was used to analyse the qPCR data.

**Results:** IHP affected gene expression of cartilage-homeostasis related targets with a large inter donor variation (figure 1). Overall, mild upregulation of FGF2 and TGFβ1 was already seen after 6h under IHP. FGF1 expression shows hardly an effect, and a mild downregulation was seen for FGF18, TIMP1 IGF1 and HGF. Interestingly, TIMP2 shows a significant downregulation for all donors.

**Conclusions:** Under IHC a mild early upregulation of FGF2, TGFβ1 and a mild downregulation of FGF18, TIMP1, IGF1 and HGF was seen. TIMP2 is the only target that shows a significant downregulation for all donors. This data suggests a first step in an early regenerative response upon IHC in hMSCs. Additional experiments are warranted to further elucidate the exact role of IHP on hMSCs.

Figure 1. mRNA expression of of cartilage-homeostasis related genes under influence of IHP.

Rehabilitation

604 PROXIMAL EFFECTS OF UNLOADER BRACING FOR MEDIAL KNEE OSTEOARTHRITIS

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**Purpose:** Persons with medial knee osteoarthritis (OA) are thought to adopt increased frontal plane trunk sway to reduce medial compartment loading. This type of compensatory motion may affect bilateral muscle function and loading of the lower extremity joints, and thereby impact risk of developing multi-articular OA. Unloading valgus knee