Purpose: To analyze the gait pattern in a mouse model of knee OA using the CatWalk systemTM, and to assess effects of intra-articular injection of hyaluronan (IAI-HA) on gait pattern.
Methods: C57BL/6 mice (9 weeks old, male) were purchased and destabilization of the medial meniscus (DMM) was performed on the left knee joint as OA knee model. In this model, OA progress slowly and it resembles slowly-progressive human OA more closely than other surgical models and allows for suitable evaluation of interventions. The CatWalk systemTM was used for gait analysis. In this system, mice were placed on glass plate runway and allowed to walk freely. The whole run was recorded by a video camera placed below the runway and gait parameters were analyzed according to foot prints by the computer automatically. To quantify longitudinal gait changes in DMM model, prior to the surgery, mice (n=5) walked on the runway 10 times for acquiring the baseline data. Then gait analysis was performed at the time point of 4, 8, 12 weeks after the induction of DMM surgery. To assess the effects of IAI-HA to the DMM model, 6 mice underwent DMM surgery, 3 underwent sham surgery where only skin incision and patella luxation was performed . Mice with DMM surgery were randomized to either treatment: (1) HA(800-kDa, ARTZ Dispo),(2) saline,3 mice for each treatment group. IAI of HA and saline were given once a week for 5 weeks both started 3 weeks after surgery. For IAI, mice were anesthetized , incision was made to identify the patella tendon and 20μL of HA or saline injection was done through the patella tendon. Gait analysis was performed for each group of mice at the time point of 3 weeks (just before IAI), 8, 12, and 16 weeks after the surgery.
Results: For each gait parameter, ratio of the affected limb to the contralateral limb was evaluated. After the DMM surgery, no significant change was seen in any gait parameter throughout the period of 8 weeks, but significant lower stand phase, single stance phase, duty cycle (percentage of stand phase during the step cycle), swing speed and significant longer duration of swing phase were observed at the time point of 12 weeks. In HA treated experiments, IAI-HA group tended to show better parameters comparing to saline group but they did not reach statistical significance even at 16 weeks.
Conclusion: Gait disturbance was detected at the time of 12 weeks after the DMM surgery. This result corresponded to the previous report that DMM progress OA slower than other surgical models. It was suggested that CatWalk systemTM could be of use to objectively quantify gait disturbance in DMM model. But we could not clarify the effects of IAI-HA on this model by gait analysis using the CatWalk systemTM.

130
APPROACHES TO TRANSCRIPTOME ANALYSIS TO STUDY JOINT REGENERATION IN THE RED-SPOTTED NEWT

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Purpose: The adult red-spotted newt *Notophthalmus viridescens* is able to regenerate damaged knee joints after local injury involving surgically induced defects, collagenase-induced joint instability, and monoiodoacetate-induced cartilage degeneration. The mechanism behind this capacity that is not present in mammals is currently not understood.
Methods: Transcriptome analysis with a custom made microarray from a normalized cDNA library was carried out after surgical and collagenase-induced knee damage in newts. Differentially expressed genes in both instances were validated by qPCR localisation studies. To improve the power of this approach, we have established a new whole newt transcriptome library using normalized cDNA from multiple regenerating and normal tissues including heart, extremities, and eyes with the 454 titanium sequencing technique. We are currently performing quantitative transcriptome analysis after knee damage with mRNA sequencing on the illumina platform.
Results: In the initial microarray analysis, a number of gene groups was found deregulated in the course of knee damage repair, most strikingly several matricellular proteins like tenascin. However, several cartilage specific genes like collagen 2 were lacking. The new library is calculated to cover the complete transcriptome with an overlap of 8 fold, annotations are currently being completed.
Conclusions: Conventional array techniques are powerful tools to study differential gene expression. However, the technique is time consuming and has a reduced power compared to the novel techniques that we are currently applying.

131
THE PROGRESSION OF MONOIODOACETATE-INDUCED ARTHRITIS INVOLVES SEQUENTIAL EXPRESSION/SUPPRESSION OF MATRIX ASSOCIATED GENES

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Purpose: Osteoarthritis (OA) is an inflammatory disease with progressive loss of cartilage and bone leading to debilitating joint pain and loss of function. Inflammation is the major cause of cartilage and bone loss. In this report, we examined the gene expression and the signaling networks associated with various stages of cartilage destruction in a rat model of monoiodoacetate-induced arthritis (MIA).
Methods: MIA was induced in the right knee joints of Sprague Dawley female rats (n=60) via intra-articular injection of monoiodoacetate (2 mg/50 μl saline). Saline (50 μl) injected knees served as sham controls. The MIA was temporally monitored macroscopically, microscopically and by μCT (micro-computed tomography) at days 5, 9 and 21 post-MIA induction, and compared to sham controls. Gene Chip analysis (Affimatrix) was utilized to analyze the transcriptome-wide changes in gene expression. The functional networks were generated by Ingenuity Pathways Analysis (IPA), and macroscopic and immunohistochemical findings were correlated with the expression of genes/gene products. Signaling pathways involved in the progression of OA were dissected to focus on salient pathways that drive the cartilage damage during the progression of MIA.
Results: The studies demonstrated that the progression of MIA was progressively damaging to cartilage and underlying bone. In this model of MIA, post-monoiodoacetate injection, Grade 1 damage was observed by day 5, which progressively increased to Grade 2 by day 9, and Grade 3 to 3.5 by day 21. The progression of MIA was accompanied by changes in gene expression, belonging to matrix synthesis/degradation. The maximally upregulated genes in the Grade 1 cartilage damage were genes involved in matrix degradation, those associated with Grade 2 damage were involved in matrix synthesis and degradation, and those associated with Grade 3 to 3.5 damage were involved in matrix synthesis. More importantly, many of these genes were those that have been identified as susceptible genes in human osteoarthritis (OA), such as Asporin,Matrixmetalloproteinase-12 (MMP-12), MMP-19, ADAMTS4, ADAMTS5, GDF5, FRZB and DIO2.
Conclusions: These findings suggest that sequential regulation of distinct gene clusters involving inhibition of matrix synthesis and induction of matrix degradation may control the progression of cartilage destruction in MIA. In this process, Asporin may act as central node regulating the processes matrix synthesis, whereas inflammatory cytokines regulate matrix degradation.

132
APPA PROVIDES DISEASE MODIFICATION IN PRECLINICAL OSTEOARTHRITIS

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Purpose: APPA, a proprietary combination of apocynin and paeonol, was evaluated for inhibition of cartilage destruction in a well-accepted rat model of osteoarthritis.
Methods: APPA is a synthetic combination of 2 molecules, 4-hydroxy-3-methoxy-acteophenone (apocynin) and 2-hydroxy-4-methoxy-acetophenone (paeonol). Male Lewis rats were anesthetized and aseptic procedures utilized to induce a medial meniscal 'tear', under an IACUC-approved protocol. APPA was orally administered at 80 mg/kg BID (n=15/group) and animals were euthanized at 3 weeks post surgery. Joints were harvested, fixed in formalin, decalcified, halved in the frontal plane, paraffin