differences were found between limbs for the external sagittal and frontal plane moments at the hip joint, subjects demonstrated greater external adduction moment at the knee of the SX side compared to the NSX side (MD: 0.12 ± 0.11 Nm/kg, p=0.013, d=1.09).

Conclusions: The Hip Harris Score, pain level, and abductor strength suggest that the SX hip is more impaired than the NSX limb. Subjects also ambulate with a compensatory lateral trunk lean toward the affected limb, but continue to demonstrate greater adduction angles on the SX limb during single limb stance. These movement abnormalities may represent the primary movement impairments and may be related to the unilateral hip abductor weakness observed in this sample. Unilateral impairments may also contribute to the significantly larger knee adduction moment on the NSX limb.

Figure 1. Average trunk lean angle (A) and hip frontal plane angle (B) during the stance phase of gait for the surgical limb (solid black line) and non-surgical limb (dashed red line). Positive angles represent lean toward the stance leg (A) and adduction (B). Black-striped area represents the standard deviation for the surgical limb. Red-squared area represents the standard deviation for the non-surgical limb.

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THE TRANSIENT EFFECTS OF COX-2 INHIBITOR ON OSTEOGENIC DIFFERENTIATION IN CANINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS
N. Oh, T. Sunaga, S. Kim, K. Hosoya, M. Okumura. Hokkaido Univ., Sapporo, Japan

Purpose: Nonsteroidal anti-inflammatory drugs (NSAIDs), especially cyclooxygenase (COX)-2 inhibitors, are simple and effective analgesics in orthopedic fields. Last decades, the effects of COX-2 inhibitor on bone healing process have remained controversial, while rare clinical data represent relationship between COX-2 inhibitor and bone healing. The aim of this study was to assess the effects of COX-2 inhibitors in canine bone marrow-derived mesenchymal stem cells (BMSCs).

Methods: Canine BMSCs were harvested from three one-year-old female beagle dogs. Osteogenic differentiation of canine BMSCs was induced by osteogenic medium containing dexamethasone (100 nM), l-glycerophosphate (10 mM), ascorbic acid (50 μg/ml) and human recombinant IL-1β (1 ng/ml) as an inflammatory stimulus. The maximum concentration of Cp (10 μM) or Mx (10 μM) that had no effect on cell viability was treated within short- and long-term of osteogenic process. The mRNA expressions of osteoblastic marker, including alkaline phosphatase (ALP) and osteocalcin, and prostaglandin E2 receptor subtypes, such as EP2 and EP4, were measured using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). For the histological evaluation, ALP staining and von Kossa staining were performed.

Results: Expression of mRNA of ALP and osteocalcin were significantly up-regulated under short-term treatment of COX-2 inhibitors. However, gene expressions of EP2 and EP4 were up-regulated under short-term treatment, and delayed osteogenic differentiation was gradually recovered after withdrawal of COX-2 inhibitors. Expression of gene was down-regulated in long-term treated group and control at the late period of osteogenic differentiation.

Conclusions: These data suggest that canine BMSCs might have potential to catch-up the osteogenic differentiation and to up-regulate osteogenic gene expression after withdrawal of COX-2 inhibitors.

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IDENTIFICATION OF FIBROBLAST GROWTH FACTOR-18 AS A MOLECULE TO PROTECT AND REGENERATE ARTICULAR CARTILAGE
Y. Mori, T. Saito, C. Ladel, H. Guerhling, U.-i. Chung, H. Kawaguchi. Faculty of Med., Univ. of Tokyo, Tokyo, Japan; Merck KGaA, Darmstadt, Germany

Purpose: Aiming at the disease-modifying treatment of osteoarthritis (OA), we sought to identify genes that maintain the homeostasis of adult articular cartilage and regenerate its lesions by gene expression profile analyses. The mechanisms of the identified molecules underlying the protection and regeneration were further investigated.

Methods: To identify genes that have both protective and regenerative effects on adult articular cartilage, we performed two sets of microarray analyses. First, to select genes that work for maintenance of articular cartilage, we compared the gene expression profiles between adult articular (AA) and adult growth plate (AG) cartilages in 10-week-old rats. Second, to find genes that function for regeneration of articular cartilage, we compared the profiles between infant superficial (IS) and infant deep (ID) layers of epiphyseal cartilage in 6-day-old rats. For genes which were up-regulated >10-fold in both AA and in IS than in ID, we performed real-time RT-PCR for the confirmation. In vivo expression was examined by immunohistochemistry of articular and growth plate cartilage of 14-week-old rats. The therapeutic effect of intra-articular injection of sprifermin (recombinant human fibroblast growth factor factor 18 (FGF18)) was examined in the experimental OA model by surgical induction of instability in the knee joints of adult rats. To further learn the underlying mechanism, the protective ability of articular cartilage was assessed by measuring the amount of sulfated glycosaminoglycan (sGAG) released into the medium in the ex vivo culture of bilateral femoral heads of 3-week-old mice using dimethyl-methylene blue dye-binding assay. Proliferation and migration were analyzed in the cultures of mouse articular chondrocytes using Cell Counting Kit-8 and Oris Cell Migration assay systems, respectively. Expression levels of catabolism-related factors (Mmp9, Mmp13, Adamts4, Adamts5, Timp1, Timp2, and Timp3) and anabolism-related factors (Col2a1 and aggrecan) in the cultures of mouse femoral heads and mouse articular chondrocytes were analyzed by real-time RT-PCR.

Results: Microarray analyses revealed that 40 and 186 genes had >10-fold higher expression ratios of AA/AG and IS/ID, respectively, and 16 genes showed >10-fold of both AA/AG and IS/ID ratios. The ratios of the 16 genes were confirmed to be >10 fold by real-time RT-PCR analysis. Among them three genes were expressed more strongly in AA than in IS. In these three genes, FGF18 was the extracellular and secreted factor of which the AA/AG ratio was the highest in the microarray analysis. Immunohistochemistry showed that FGF18 was strongly expressed in the articular cartilage chondrocytes of adult rats but was hardly detected in the growth plate cartilage. In the rat surgical OA model, a once-weekly injection of sprifermin given 3 weeks post-surgery prevented cartilage degeneration in a dose-dependent manner at 6 and 9 weeks after surgery, with a significant effect at 10 μg/week of sprifermin. As an underlying mechanism, sprifermin suppressed the sGAG release into the culture medium in the ex vivo culture of mouse femoral heads. Furthermore, sprifermin accelerated proliferation and migration of cultured mouse articular chondrocytes. Among catabolic and anabolic factors, sprifermin decreased Adamts4 and increased Timp1 expressions in the cultures of mouse femoral heads and murine articular chondrocytes; however it decreased Col2a1 and aggrecan expression in both cultures.

Conclusions: The present gene expression profiling analysis identified sprifermin as a molecule to protect and regenerate adult articular cartilage, causing prevention of cartilage degeneration by the once-weekly intra-articular injection in a rat model. This effect may be due to inhibition of cartilage catabolism, and acceleration of proliferation and migration of articular chondrocytes, providing a disease-modifying OA treatment.

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OLD AND YOUNG ARTICULAR CARTILAGE RESPOND EQUIVALENTLY FOR PHYSIOLOGICAL AND EXCESSIVE LOADING BY ACTIVATION OF PROTEINASE TGF-BETA SIGNALING

Purpose: Old and young articular cartilage respond equivalently for physiological and excessive loading by activation of transcription factor TGF-beta signaling.
Purpose: (Partial) Meniscectomy causes dramatic changes in knee joint biomechanics. As a consequence, this surgical intervention generally leads to the development of osteoarthritis (OA). Furthermore, the majority of current evidence shows that age is one of the most important risk factors for OA development after meniscectomy. However, the mechanisms how changed loading patterns lead to OA and how age related changes in articular cartilage influence its response for increased loading remains largely unknown.

Our previous findings showed that in young and healthy articular cartilage, when subjected to compression, TGF-beta signals via type 1 receptor ALK5 causing activation of downstream genes specific for Smad2/3 signaling pathway. Since in aged articular cartilage there is loss of type ALK5 leading to shift towards Smad1/5/8 signaling pathway, we wanted to investigate how TGF-beta signals in aged articular cartilage when subjected to physiological and excessive loading.

Methods: Full-thickness articular cartilage specimens were cored from metacarpophalangeal joint surface of two different age groups of cows (juvenile of 1 year old and aged of 8-12 years old). All explants were allowed to equilibrate for 48 h in static, serum free culture conditions. Explants were divided into two stimulation groups: 30 min stimulation with 3 MPa (physiological load) and 30 min stimulation with 12 MPa (excessive load). Unloaded cartilage was used as controls. Stimulation groups were subjected to sinusoidal compression (1 Hz) using BOSE ElectroForce® BioDynamic® test system, in culture conditions. After 2 and 6 hours cartilage samples were frozen and mRNA levels of selected genes were examined using real-time polymerase chain reaction (RT-PCR).

Results: In 2 hours after the stimulation there was significant upregulation of TGF-beta1 in both age groups when stimulated with 3 MPa but also with 12 MPa compared to the static control. However, in young articular cartilage this upregulation was more pronounced than in old. In 6 hours after the stimulation, in young cartilage, TGF-beta was upregulated in control group (when compared to 2 hours time point), therefore the effect of stimulation was less pronounced. This was not the case in aged cartilage where in both stimulation groups TGF-beta was upregulated when compared to static control.

Examination of TGF-beta signaling pathways downstream genes expression demonstrated that in 2 hours after the stimulation in both age groups, 3 MPa and 12 MPa stimulation caused upregulation of P-A1. The level of upregulation was higher in young articular cartilage but this could be related to lower P-A1 expression in static controls of young cartilage. In aged articular cartilage, P-A1 upregulation was caused only by 12 MPa stimulation. Stimulation with both physiological (3 MPa) and excessive loading (12 MPa) showed no effect on ID1 expression when measured in 2 hours in both age groups. However in 6 hours after the stimulation in 12 MPa compression group ID1 was down-regulated in both age groups.

Conclusions: Unexpectedly many changes in expression of genes caused by repetitive loading did not depend on the amount of force that was applied in our experiments. Mechanical compression with physiological and excessive forces affected similar TGF-beta-signaling related genes in young and old cartilage, however, in young cartilage with a greater magnitude. Although ALK5 expression is decreased in aged articular cartilage, loading is still able to activate P-A1, indicating activation of the Smad2/3 signalling pathway. This indicates similar but not equal stimulation of TGF-beta signaling in different age groups. Since activation of protective TGF-beta signaling by loading is higher in young cartilage this might indicate that the protective effects of loading are more pronounced in young cartilage than in old cartilage.

188 AMINO ACID RACEMIZATION REVEALS A HIGH STATE OF REPAIR IN KNEE COMPARED WITH HIP OSTEOARTHRITIC CARTILAGE

J. Catterall, R. Zura, M. Bolognesi, V. Kraus. Duke Univ., Durham, NC, USA

Purpose: After synthesis, proteins undergo age dependent non-enzymatic post-translational modifications such as amino acid racemization and protein oxidation. Many cartilage proteins have long half-lives, so are prone to accumulation of post-translational modifications with age. During previous studies we quantified protein racemization in whole cartilage; osteoarthritic (OA) cartilage appeared to accumulate racemized amino acids at a slower rate than normal cartilage suggesting higher turnover of cartilage with OA. Our recent work with deaminated COMP showed greater accumulation of this post-translationally modified protein in hip OA than knee OA suggesting higher turnover of cartilage extracellular matrix in knee joints. The goal of this study is to confirm and expand upon our earlier investigations of protein turnover in health and disease through investigation of protein racemization in soluble (mainly aggrecan) and insoluble (mainly collagen) cartilage fractions of knee and hip non-OA and OA joints.

Methods: With IRB approval, cartilage was collected at the time of joint replacement for OA or trauma surgery. OA tissue was split into macroscopically lesioned and normal (remote from lesion) regions. Cartilage was pulverized and extracted in 4M guanidine HCl to isolate soluble proteins; the extracted residual cartilage represented the insoluble protein fraction. Samples were hydrolyzed in 6M HCl, fluoro- rescently labeled and both the non-racemized (L) and the racemized (D) forms of the amino acids Asx (Asp and Asn) and Ser were quantified using reverse phase HPLC. Data were expressed as a ratio of D/(D+L). The association of racemized protein with age was determined by linear regression; statistical differences in racemized protein content between joint sites was analyzed using a multivariable model with age as a covariate.

Results: In non-OA cartilage amino acid racemization was associated with age: insoluble Asx (p=0.0006, r²=0.61) and Ser (p=0.01, r²=0.39) and a trend for soluble Asx (p=0.05, R²=0.35). Remote OA cartilage from both joint sites showed similar associations with age: insoluble Asx (p=0.0002, R²=0.32) and Ser (p<0.0001, R²=0.40) and a trend for soluble Asx (p=0.06, R²=0.09). Lesioned OA cartilage however only demonstrated a weak association with age based on insoluble Ser (p=0.03, R²=0.16). While hip OA fractions demonstrated significant associations of racemized amino acids with age, no significant associations of age for any of the knee fractions: hip remote insoluble Asx (p=0.06, R²=0.19) and Ser (p=0.01, R²=0.34), lesion soluble Asx (p=0.03, R²=0.66) and Ser (p=0.04, R²=0.62). Accounting for age, hip had a significantly higher content of racemized amino acids compared with knee: remote insoluble Asx (p=0.0001) and Ser (p=0.0001), remote soluble Asx (p=0.001).

Conclusions: Our data on OA lesional cartilage demonstrates a loss of the normal age-related increase in racemization that is particularly striking for the knee. Compared with knee, the hip soluble and insoluble cartilage fractions had a higher content of racemized amino acids. Taken together, these data strongly suggest that protein turnover increases in OA, particularly at sites of OA lesions. These data further demonstrate that hip OA cartilage turns over more slowly than knee OA cartilage; conversely, these data are compatible with a less robust reparative anabolic response in hip OA compared with knee OA. These results suggest that effective treatment of hip OA may require anabolic stimuli in addition to anti-catabolic stimuli. Effective treatment of knee OA may require mainly anti-catabolic treatment strategies given the apparent upregulating evidence of the ongoing repair and high turnover state in knee OA.

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