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=1.095).

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.

185 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 on osteogenic differentiation of 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), β-glycerophosphate (10 mM), ascorbic acid (50 μM) and human recombinant IL-1β (1 ng/ml) as an inflammatory stimulator. 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, involving alkaline phosphatase staining, were measured using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). For the subtypes, such as EP2 and EP4, were measured using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The mRNA expressions of osteoblastic marker, involving alkaline phosphatase staining, were measured using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The mRNA expressions of osteoblastic marker, involving alkaline phosphatase staining, were measured using quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Results: Expressions of mRNA of ALP and osteocalcin were significantly suppressed by use of COX-2 inhibitors. Alkaline phosphatase staining revealed that COX-2 inhibitors partially arrested early differentiation of osteoblast lineage. However, gene expressions of EP2 and EP4 were up-regulated under COX-2 inhibition, and delayed osteogenic differentiation was gradually recovered after withdrawal of COX-2 inhibitors. Calcification of matrix showed no significant difference between short- 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.

186 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. 1 Faculty of Med., Univ. of Tokyo, Tokyo, Japan; 2 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 molecule 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 both in AA than in AG 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, Admats4, Admats5, 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 Admats4 and increased Timp1 expressions in the cultures of mouse femoral heads and murine articular chondrocytes; however it decreased Col2a1 and aggrecan expressions 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.

187 OLD AND YOUNG ARTICULAR CARTILAGE RESPOND EQUIVALENTLY TO PHYSIOLOGICAL AND EXCESSIVE LOADING BY ACTIVATION OF PROTECTIVE TGF-BETA SIGNALING