120 EXTRACELLULAR MATRIX CHANGES IN RESPONSE TO RECOMBINANT HUMAN FIBROBLAST GROWTH FACTOR 18 STUDIED IN EX VIVO CULTURES OF ARTICULAR CARTILAGE

D. Reker, T. Christiansen, M. Karsdal, A. Bay-Jensen, Nordic BioSci. A/S, Herlev, Denmark; Orthopedic Dept., Gentofte Univ. Hosp., Hellerup, Denmark

Purpose: Osteoarthritis (OA) is a degenerative disease with high prevalence, creating an unmet medical need for drugs to regenerate cartilage. A promising candidate for a novel disease modifying osteoarthritis drug (DMOAD) is Sprifermin, a truncated form of fibroblast growth factor 18 (FGF18). Sprifermin has been demonstrated to increase cartilage volume in the knees of OA patients, but surprisingly little is known about the mode of action behind its anabolic effects. The few studies published indicate that full-length FGF18 induces cartilage formation by increasing chondrocyte proliferation, resulting in an increased overall matrix production by the larger population of chondrocytes. Our hypothesis is that matrix degradation is initially needed during this process in order to expand the lacunae and make room in the matrix for the new chondrocytes. Accordingly, the aim of this study was to characterize the changes in matrix degradation occurring in response to direct stimulation with recombinant human (rh) FGF18.

Methods: Full depth cartilage explants (FDCex) punched from bovine articular cartilage were cultured for 27 days. In replicates of six, the FDCex were treated with various concentrations of full-length rhFGF18 (1, 10, 50, 100 or 500 ng/mL rhFGF18), an anabolic cytokine as positive control for cartilage formation (100 ng/mL IGF-I), or culture media without treatment as negative control (W/O). Supernatants were harvested and replaced 3 times weekly. Cell viability was measured using AlamarBlue at day 27. Biomarkers released to the supernatant were measured using the following well-described ELISA: C2M and AGNx2 reflecting matrix metalloproteinase (MMP)-mediated degradation of type II collagen and aggrecan, respectively, AGNx1 reflecting aggrecanase-mediated degradation of aggrecan, and C-Col10 reflecting chondrocyte hypertrophy. Mean values and standard error of the mean (SEM) were compared using one-way ANOVA assuming normal distribution. Significance levels are indicated by asterisks; *P < 0.05, **P < 0.01.

Results: To evaluate the changes in matrix degradation occurring in the FDCex in response to direct stimulation with rhFGF18, three different biomarkers of matrix degradation were quantified (figure 1). According to C2M, MMP-mediated type II collagen degradation is significantly decreased in response to > 50 ng/mL rhFGF18 from day 11 onward (P < 0.05). Likewise, AGNx2 indicates a slight decrease in MMP-mediated aggrecan degradation in response to > 50 ng/mL rhFGF18 from day 18 onward, although not significant. On the contrary, AGNx1 reveals increased aggrecanase-mediated aggrecan degradation in response to > 10 ng/mL rhFGF18 at day 25, although only significant for 50 ng/mL rhFGF18 (P < 0.05). Evaluation of the chondrocytic changes occurring in response to rhFGF18, reveal a dose-dependent increase in cell viability measured at day 27, and no indication of hypertrophic cell differentiation, as assessed by C-Col10.

Conclusions: The data presented here indicate that direct stimulation of articular cartilage with rhFGF18 leads to decreased generation of MMP-mediated cleavage fragments of type II collagen and aggrecan, but increased generation of the aggrecanase-mediated cleavage fragment of aggrecan. Interestingly, AGNx2 is believed to be a marker of cartilage degradation with impaired repair capacity, whereas AGNx1 is considered a marker of reversible form cartilage degradation. Accordingly, the increased AGNx1 could indicate the matrix degradation needed for expansion of the lacunae, which according to our hypothesis is needed to initiate the process of cartilage formation. Clarifying the steps of this process is highly important for the understanding of how a potential novel DMOAD is affecting the tissue.
posterior view radiograph of painful knee were measured at baseline and 3 years later. Experienced readers read the radiographs independently recorded the radiographic features of Kellgren & Lawrence grading (K/L) grade and joint space width (JSW). JSW was determined at the center point of the medial femoro-tibial compartment on a radiograph using a 0.1-mm graduated magnifying lens. Both serum and urine samples were obtained on the day that baseline radiographs taken. Measurement of uCTX-II was used commercial enzyme-linked immunosorbent assay kit (CartiLaps; Nordic Bioscience, Herlev, Denmark). uCTX-II values were corrected for urine creatinine concentration. As the distribution of uCTX-II was found to be positively skewed, a logarithmic transformation (natural log (Ln)) was therefore applied to these biomarkers to obtain an approximately normal distribution. uCTX-II were compared using parametric comparisons analysis of variance (ANOVA). The Bonferroni correction for multiple comparisons was applied. Significant differences were evaluated t-test if ANOVA was significant. A P-value of less than or equal to 0.05 was considered to be statistically significant.

**Results:** Of the 91 patients, 7 showed K/L grade 1, 16 showed K/L grade 2, 27 showed K/L grade 3 and 41 showed K/L grade 4 at the baseline. In the 3 years, 41 patients were received total knee arthroplasty (TKA). In the other patients, 22 patients were progressed their K/L grade one or more. The ln-uCTX at baseline was not significant differences between K/L grades. Among the patients with K/L grade 1 to 3, the baseline ln-uCTX-II levels in the patients with progression of K/L grade were significantly higher than the patients with no progression of K/L grade (p = 0.002).

**Conclusions:** We demonstrated in the present study that the patients who were progressed OA present by K/L grade during 3 years had a higher baseline ln-uCTX-II, cartilage degradation marker, levels than the others. These findings suggest that we could predict OA progression at the first visit in the hospital by using biomarkers. In conclusion, uCTX-II could predict OA progression.

### 122 COLL2-1NO2: A BIOMARKER FOR EARLY KNEE OSTEOARTHRITIS?


**Purpose:** To investigate the association between urinary degradation biomarker Coll2-1NO2 (uColl2-1NO2) and incident knee OA after 2.5 years in overweight middle-aged women at high risk for knee OA. Secondly, to assess association of uColl2-1NO2 with cartilage loss progression on magnetic resonance imaging (MRI).

**Methods:** Data were used from PROOF, a 2.5 years randomized controlled trial evaluating the preventive effects of a diet and exercise controlled, on development of knee OA in women with body mass index ≥ 27 kg/m2 and without knee OA at baseline. Baseline, 1 and 2.5 years uColl2-1NO2 levels were assessed by ELISA. Primary outcome measure was incidence of knee OA in one or both knees, defined as incidence of either Kellgren & Lawrence grade ≥ 2, joint space narrowing of ≥ 1.0 mm or clinical knee OA according to the combined clinical and radiographic ACR-criteria. Secondary outcome measures were the separate radiographic and clinical items and the MRI progression of cartilage loss in the patellofemoral and tibiofemoral joint, using the MRI Osteoarthritis Knee Score (MOAKS). Association between baseline, follow-up and change of uColl2-1NO2 with primary and secondary outcomes were assessed using linear logistic regression analyses.

**Results:** 254 women were available for primary analysis, 234 women for MRI analyses. After 2.5 years follow-up, knee OA developed in 72 of 254 women (28.3%) and progression of cartilage defects in 115 of 234 women (49.1%). An inverted association was found between baseline uColl2-1NO2 and incident knee OA (OR 0.74, 95% CI 0.55 – 0.99) after 2.5 years follow-up. A trend of increasing uColl2-1NO2 during follow-up of 2.5 years was found in incident knee OA and in progression of cartilage defects (OR 1.10, 95% CI 0.81 – 1.48 and OR 1.26, 95% CI 0.93 – 1.70 respectively). This trend of increasing uColl2-1NO2 is also shown in the figure.

**Conclusion:** In overweight middle-aged women, low baseline uColl2-1NO2 levels were associated with an increased risk for incident knee OA. We can propose different hypothesis to explain this finding: 1) an anabolic compensatory mechanism active in early knee OA, 2) in contrast, a decrease of cartilage metabolism could precede cartilage degradation; 3) cartilage volume is lower in people developing knee OA. Overall, after lower baseline uColl2-1NO2, increasing uColl2-1NO2 levels seem to dominate in further OA development. Although in early knee OA changes in uColl2-1NO2 can be found, further exploring of the biomarker field seems necessary to better understand the onset of OA in high risk patients.

### 123 CORRELATION ANALYSIS OF INFLAMMATION BIOMARKERS WITH CLINICAL SCORES OF TISSUEGENE-C (TG-C)


**Purpose:** Osteoarthritis (OA) is a degenerative joint disease caused by unbalanced anabolism and catabolism of cartilage with a mild inflammatory response, but little is known about how the level of inflammation is related to severity of OA symptom and tissue destruction. The level of circulating acute phase protein, C-reactive protein (CRP) has been widely used as a diagnostic marker in acute inflammatory diseases. The matrix metalloproteinase-mediated C-reactive protein (CRPM) is a hallmark of chronic tissue inflammation in the knee joint of OA patients. TG-C is a cell mediated gene therapy product that contains non-transduced (hChon) and transduced (hChonβG7) human algogenic chondrocytes. The hChonβG7 cells were transduced with TGF-β1 gene-containing retroviral vector. The aim of this study was to evaluate correlation between inflammatory biomarkers in OA patients and the changes of clinical scores of IKDC and WOMAC to evaluate the prognostic feature for this TG-C therapy.

**Methods:** Data from clinical scores of IKDC, WOMAC, IKDCα, the changed value of IKDC score evaluated 6 months post treatment of TG-C and WOMACα, the changed value of WOMAC score evaluated 6 months post treatment of TG-C were collected from patients during TG-C clinical trials, phase IIa (KS-TGC-01-2a) and phase IIb (KS-TGC-01-2b). The patients enrolled in the clinical trials of TG-C included advanced joint damage (K&L grade 3, IRCS grade IV in main cartilage lesion) and various range of knee clinical scores (Table 1). The level of serum high sensitive CRP (hsCRP) and CRPM were detected by enzyme linked immunosorbent assay (ELISA). The values were compared between studies and treatment groups within each study by a non-

---

*Abstracts / Osteoarthritis and Cartilage 22 (2014) S57–S589*