serum level of the bone turnover marker osteocalcin was significantly (P < 0.001) elevated in OVX and PTH treated compared to sham and vehicle treated, while the collagen type II degradation decreased, as the level of serum cartilage degradation marker CTX-II was reduced by 30% (P < 0.01), compared to vehicle treated OVX animals.

Conclusions: Human OA chondrocytes express the PTHR1 receptor and PTH seems to induce an anabolic response in articular cartilage. Moreover, PTH had an anti-catabolic effect in the preclinical model of accelerated cartilage loss. Current results strongly suggest that PTH has direct beneficial effects on human OA chondrocytes and cartilage. Further research is necessary to investigate the potential of PTH as a disease modifying OA drug (DMOAD).

Materials and Methods: OA articular cartilage was obtained from femoral condyles of patients with medial type OA during total knee arthroplasty. Lateral condyles and medial condyles were used as mild OA cartilage and severe OA cartilage respectively. Human non-OA cartilage was obtained from femoral heads of patients with femoral neck fracture and used as non-OA cartilage. Primary chondrocytes were also isolated from the articular cartilage samples of patients with OA and used as OA chondrocytes. Normal Human Articular Chondrocytes-knee (NHAC-kn) cells were purchased and used as normal human chondrocytes. The state of autophagy in the articular cartilage samples and the chondrocytes were assessed by immunohistochemistry and immunoblotting using antibodies for autophagy markers, LC3II and beclin1. In addition, we stimulated NHAC-kn cells with various stresses (IL-1β, NO, serum starvation) and examined the effects of the stresses on the autophagic activity by real-time PCR and immunoblotting.

Results: Immunohistochemical analysis showed that the expression of LC3 and beclin-1 were increased in the mild OA cartilage compared with the non-OA cartilage. On the other hand, the expression of LC3 and beclin-1 were decreased in the severe cartilage. LC3 and beclin1 were more strongly expressed in the superficial zone of the mild OA cartilage than in the deep zone and the middle zone. In addition, the primary OA chondrocytes strongly expressed LC3II and beclin-1 compared with NHAC-kn. Furthermore, the expression of LC3 and beclin-1 in NHAC-kn were increased by the stresses.

Conclusions: In this study, we found that autophagy was increased in the mild OA cartilage. Autophagy was especially increased in the superficial zone presumably where chondrocytes were under more stresses compared with other zones. In addition, autophagy was increased in normal chondrocytes by the stresses in vitro. These observations suggested autophagic activity increases during early stage of OA and the increased autophagy was an adaptive response to protect cells from stresses. On the other hand, autophagy was decreased in the severe OA cartilage, suggesting that dysfunction of autophagy might be a cause of the progression of OA. Further studies about autophagy in chondrocytes will provide novel insights into the pathophysiology of OA.
mechanical stress-induced matrix synthesis was completely cancelled by application of p38 pathway inhibitor, whereas inhibition of ERK or JNK pathway resulted in incomplete attenuation of matrix synthesis.

**Conclusions:** The present results show that MS influence the matrix synthesis of the 3D-embedded chondrocytes. In turn, the inhibition of the mechanical stress-related enhancement of matrix synthesis by the p38 inhibitor strongly suggests that p38 mediates an intracellular pathway, the role of which is prevalent in the 3-dimensionally intact environment.

**259 PLASTIC-ADSORBED ALTERNATIVELY SPliced SEGMENTS AND PARTIAL ISOFORMS OF FIBRONECTIN DIFFERENTIALLY AFFECT APOPTOSIS AND VIABILITY OF MONOLAYER-CULTURED RCJ 3.1C.518 RAT CHONDROCYTES**

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**Purpose:** Given the restricted expression of the alternatively spliced EIIIA and V segments of fibronectin (FN) in cartilage, we wished to determine if extracellular environments containing either of these segments exert effects on viability, apoptosis, or proliferation of adherent chondrocytes.

**Methods:** We tested the effects of EIIIA, V and III-10 segments of FN, expressed in isolation or in the context of partial FNs spanning the 7th through 15th type III repeats (III7–15 FNs), coated on plastic substrates, on RCJ3.1C.15B (C5.15) chondrocytes grown in monolayer culture in the absence of serum. Five III7–15 FN constructs were studied, differing in inclusion of the RGD sequence in III-10 ("R+" with RGD included and "R-" with RGD excluded), as well as EIIIA and V segments ("A+" including EIIIA, "V+" including V, and "SM" including no spliced segments, or "splice minus"). Assessment of the number of viable adherent cells at time points was made by MTT assay, whereas assessment of the percentage of attached cells undergoing apoptosis was made by aspartic acid-rhodamine-aspartic acid (D2R) caspase substrate fluorescent assay.

**Results:** In 15% Knockout Serum Replacement (KSR)/85% alpha-MEM, significantly greater numbers of viable attached chondrocytes remained in wells coated with III-10 (at 1, 2, 3, and 7 days), V (at 1 day) and EIIIA (at 1 day) compared to uncoated wells. Significantly greater numbers of cells also remained attached to III7–15 FN-coated surfaces in comparison to uncoated wells on days 1 (R+SM, R+A+), 2 (R+SM and R+A+), 3 (R+SM) and 7 (R+SM). Despite the presence of an RGD sequence in both III-10 and V, wells coated with R+SM contained more viable attached chondrocytes than wells coated with R+V on all days. At an early time points (4 h), in KSR medium with 100 μM manganese (Mn), numbers of DAPI-stained cells remaining adherent to the EIIIA, V or III-10 segments were 95.0±103.6, 179.7±5.8, 197.3±18.8, whereas numbers for R+V, R+, V+ and R+SM, and R+SM partial FNs were 151.3±13.9, 24.7±38.4, 143.3±53.5, 160.7±23.7, and 112.7±28.4. No significant differences in adhesion were observed between the three FN segments, but R+A+ promoted significantly less adhesion than the other 4 partial FNs. Among adherent chondrocytes, the percentage (%) that were apoptotic at 4 h were 72.5±35.9, 29.8±17.8 and 25.2±7.8 for the isolated EIIIA, V and III-10 segments. Corresponding values for R+A+, R+V, R+V+, R+SM, and R+SM III7–15 FNs (the low number of cells adherent to R+V+ precluded assessment of apoptosis), were 23.8±6.6, 30.9±14.7, 26.5±12.6, and 72.7±7.8. Values for the three isolated segments did not differ significantly but, among the III7–15 partial FNs, R+SM promoted significantly greater apoptosis of adherent cells than the other three constructs.

**Conclusions:** The loss of viable adherent monolayer-cultured chondrocytes over time in the absence of serum can be reduced on plastic substrates coated with isolated III-10, V, or EIIIA segments, in order of effectiveness. Similar effects are exerted by III7–15 partial FNs, with an order of effectiveness: R+SM, R+A+, R+V, R+A- and R+SM. Therefore, both RGD sequences of rat FN (in the 10th type III repeat and in the V segment) play roles in the maintenance of viable attached chondrocytes in serum-free culture. However, the RGD in III-10 produces a significantly stronger effect than the corresponding sequence in V. In the presence of Mn, partial FNs encompassing the 7th through 15th type III repeats promote chondrocyte adhesion even without inclusion of RGD motifs. However, this occurs in association with significantly higher apoptosis than with R+ III7–15 FNs.