Conclusions: Both thrombin and plasmin were able to induce proteoglycan release in a dose-dependent manner. PAR1 and PAR2 antagonists were not able to inhibit the thrombin- and plasmin induced proteoglycan release. Furthermore, the PAR agonists TFLLR-NH2 and SLIGKV failed to induce proteoglycan release. This suggests that both thrombin and plasmin induce proteoglycan release and that both are able to damage the human cartilage in a PAR-independent manner.

215 STUDY OF THE ASSOCIATION BETWEEN BONE SIALOPROTEIN, HYPERTROPHIC DIFFERENTIATION OF CHONDROCYTES AND CARTILAGE LESIONS IN OSTEOARTHRITIC CARTilage

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Purpose: Chondrocyte hypertrophy is commonly observed in OA cartilage, associated with matrix mineralization and vascularization. In previous work, we demonstrated that hypertrophic differentiation of chondrocytes is initiated by serum-enriched medium in long-term culture in alginate beads suggesting a role played by blood supply in the hypertrophic differentiation of chondrocytes in OA.

This study aims to investigate the production of Bone Sialoprotein (BSP), an angiogenesis enhancer known to promote endothelial cell attachment and migration, during hypertrophic differentiation of OA chondrocytes and its expression in OA cartilage according to the severity of the lesions by immunohistochemistry. This work was supported by an OARSI scholarship grant.

Methods: Articular OA chondrocytes were cultured for 28 days in alginate beads in culture medium containing 2% Ultrasor G (UG) or 10% Fetal Bovine Serum (FBS). DNA was quantified by fluorimetry. The expression of BSP and hypertrophic differentiation markers COL10A1 was evaluated by RT-PCR. Alkaline phosphatase (AP) activity and 5′-phosphodiesterase activity of NTTPPH were quantified by specific enzymatic methods. Western Blot analysis was performed from chondrocyte protein extracts with anti-BSP antibody (LFBM-24).

Human bone and cartilage samples from 9 post-mortem (PM) individuals and 24 patients undergoing total knee joint replacement for OA (TKR) were formalin-fixed, EDTA-decalcified and wax embedded. Five 10μm tissue sections were cut and stained. Macroscopic chondrocyte score described previously by Walsh et al. and the modified Mankin score were respectively used to establish macroscopic and microscopic chondrocyte scores for individual knees. BSP was immunolocalized in cartilage using anti-BSP monoclonal antibody (LFBM-25) and anti-BSP polyclonal antibody (ab52128) to confirm the specificity of staining. A scoring system was established according to the location of the stained cells in the middle layer - 1: staining in the deep layer - 2: staining in the middle layer – 3: staining in the deep layer. All histological scoring was undertaken twice blinded to patient group, using a Zeiss Axioscop-50 microscope.

Data were analyzed using GraphPad Prism software, version 5. Associations between variables were reported as Spearman’s rank correlation coefficients (r).

Results: In alginate beads, chondrocytes cultured in serum-supplemented medium underwent a hypertrophic differentiation process characterized by the increased expression of hypertrophic differentiation markers. In the same manner, the expression of BSP increased in FBS with long-term culture and was associated with markers of chondrocyte hypertrophy; COL10A1 (r = 0.67; p = 0.0005), AP (r = 0.8; p = 0.0002) and NTTPPH (r = 0.68; p = 0.004). After 21 days, BSP was also detected in protein extracts of chondrocytes cultured in serum but not in UG. BSP-immunolocalization was characterized by an increased expression in cartilage from OA joints (2.3 ± 0.63) than from PM controls (0.56 ± 0.54). The expression of BSP in patients with OA was associated with the severity of macroscopic cartilage lesions (r = 0.5; p = 0.01). Highly significant correlations were also observed with the modified Mankin score (r = 0.71; p < 0.0001) and with the individual scoring criteria of cartilage surface integrity (r = 0.51; p = 0.01), chondrocyte appearance (r = 0.44; p = 0.03) and proteoglycan loss (r = 0.61; p = 0.001).

Conclusions: Genetic and protein expression correlation of BSP are associated with hypertrophic differentiation of chondrocytes in OA. Location of BSP in OA cartilage is clearly associated with macroscopic and microscopic cartilage lesion severity. BSP may be an important factor in cartilage degradation and its role as an angiogenesis enhancer in OA is still to be demonstrated.

216 COMMON AND EARLY CARTILAGE DEGENERATION PATTERNS IN POST-TRAUMATIC AND OSTEOARTHRITIC HUMAN KNEES

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Purpose: A better understanding of early tissue degeneration patterns in the aging knee can help to find new targets for osteoarthritis (OA) prevention and treatment. The objective of this study was to identify common and early morphologic patterns of cartilage degeneration during human joint aging, and to establish their correlation with changes in anterior cruciate ligament (ACL), meniscus, and synovium.

Methods: 130 human knees (67 donors, age range 23–96), obtained from tissue banks, were divided into eight age groups: 21–30 (n = 8), 31–40 (n = 6), 41–50 (n = 18), 51–60 (n = 21), 61–70 (n = 22), 71–80 (n = 20), 81–90 (n = 26) and 91 (n = 9). All cartilage compartments were macroscopically assessed according to the ICRS mapping system by using a modified Outerbridge grading system. Menisci and ACL were evaluated macroscopically and by histopathology. Synovium histopathology was assessed using a modified Krenn grading system for chronic synovitis. Osteophytosis was macroscopically assessed.

Results: We found a strong correlation between cartilage, meniscus, and ACL degeneration with aging. The earliest cartilage lesions were detected in the central area of the lateral tibial plateau that is not covered by the meniscus, followed by changes in patella and trochlea. Degeneration in the medial femoral condyle appeared later than in the tibia plateau but progressed rapidly to become the most severely affected among all articular surfaces. On the other hand, degeneration in the lateral femoral condyle progressed less rapidly in all age groups. In the lateral TFJ, more knees had a higher tibial than femoral average grade at all ages. This pattern was similar but reversed with aging in the medial TFJ. Degeneration of the meniscus-covered tibia surface correlated with increased meniscus degeneration. A common macroscopic pattern (33%) was degeneration in tibia and meniscus with a normal-appearing femoral condyle. In 14% we found degenerative changes in the tibia while menisci and femoral condyles were normal. In 18% we found a macroscopically normal meniscus while there were degenerative changes in tibia (uncovered portion) and femoral condyle. ACL scores increase with cartilage degeneration at all stages of OA development and correlated with cartilage as well as meniscus degeneration, especially in the medial compartment. Moderate and severe OA groups showed significantly higher ACL substance scores than the minimal/mild OA groups. Synovitis correlated with cartilage degeneration and the presence of osteophytes. In our donor population every knee with osteophytes was accompanied by synovitis.

Conclusions: By examining younger age groups, we were able to identify early patterns of cartilage degeneration. Our data suggest that in younger subjects the lateral tibia plateau shows the earliest and most severe degeneration. Contrary to the notion that the medial TFJ compartment in severe OA is most affected, we found that the lateral tibia plateau is affected the earliest. The uncovered part of the tibia plateau degenerated first, followed by early surface degeneration in the inner rim of the meniscus. Degeneration of the covered tibia region increased with cumulative meniscus degeneration, supporting the protective role of menisci. This cross-sectional study emphasizes the importance of interaction of the different joint tissues in OA initiation and progression and defines specific regions in the joint for future studies on biomechanical and cellular factors that determine early stages of OA initiation to reveal novel targets for preventing or delaying OA.

217 COMPARISON OF SYNOVIAL FLUID ARGs CONCENTRATIONS AT BASELINE AND ONE-YEAR POST-ACL RECONSTRUCTION COMPARED TO HEALTHY, MATCHED CONTROLS

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Purpose: The clinical signs and symptoms of post-traumatic osteoarthritis (PTOA) following severe joint injury, such as that associated with anterior cruciate ligament (ACL) tears, typically present 10–20 years after injury. However, the mechanism of how this occurs is still not well understood. In this study, we investigated whether acute deformation of cartilage, i.e., ACL injury, could enhance the expression of acid and proteinases (ARGs), which promote cartilage degradation in OA.