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ELEVATED LDL CHOLESTEROL-LEVELS DURING EXPERIMENTAL OA LEADS TO INCREASED SYNOVIAL THICKENING, S100A8/9 PRODUCTION AND ECTOPIC BONE FORMATION

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Purpose: In a previous study, we showed that LDL accumulation by LDL receptor deficient mice resulted in increased ectopic bone formation during experimental osteoarthritis (OA). Furthermore, we found that S100A8/A9 proteins are crucial in mediating joint pathology during experimental OA. In the present study we investigate OA pathology and its correlation with S100A8/9 in ApoE deficient (ApoE−/−) mice, which is a different model for studying effects of systemically high LDL cholesterol levels.

Methods: Wild type (WT) and ApoE−/− mice received a normal or cholesterol-rich diet for 54 days. At day 18, experimental OA was induced by intra-articular injection of collagenase and animals were sacrificed at day 28 and 54. Synovial RNA expression and joint pathology was investigated by RT-PCR and histology, respectively. LDL and S100A8/9 levels were measured in serum and synovial wash-outs.

Results: ApoE−/− mice on a normal diet showed remarkably higher LDL levels than WT mice (8.90 mmol/L and 0.40 mmol/L, respectively; p < 0.0001). Experimental OA in ApoE−/− mice showed no increase in synovial thickening and ectopic bone formation, but a significant increase of cartilage damage was found in ApoE−/− mice compared to WT mice at the lateral side of the femoral condyle (OARS score 13.7 and 6.8, respectively; p < 0.05). Synovial gene expression of both S100A8 and S100A9 was significantly increased in ApoE−/− mice compared to WT mice (fold increase 1.8 and 1.4, respectively; p < 0.05). Furthermore, S100A8/S100A9 protein levels of synovial wash-outs was increased in ApoE−/− mice at day 28 (fold increase 5.8; p < 0.05), which was confirmed by immunohistochemical staining for S100A8. In addition, we investigated whether a cholesterol-rich diet could increase joint pathology after induction of OA. A cholesterol-rich diet increased differences in LDL levels even more (18.4 mmol/L in ApoE−/− mice versus 1.2 mmol/L in WT mice; p = 0.0001) and already 10 days after induction of OA, histological differences between the two groups were observed. Synovial thickening was increased by 400% in ApoE−/− mice compared to WT mice (p < 0.001) and also ectopic bone formation in the medial collateral ligament was strongly increased at this early time point (fold increase 2.7; p < 0.01). Cartilage damage, however, was comparable to cartilage damage observed in mice on a normal diet. Again, 36 days after induction of OA, S100A8/S100A9 levels were strongly increased in ApoE−/− mice, both on protein and gene expression level and significantly correlated with ectopic bone formation. Ectopic bone formation in the medial collateral ligament at this time point was massive in ApoE−/− mice on a cholesterol-rich diet (figure 1).

Conclusions: LDL cholesterol accumulation by ApoE–/– deficiency results in increased both S100A8 and S100A9 production by synovial cells. A cholesterol-rich diet further increases this production which correlates with increased synovial thickening and ectopic bone formation in experimental OA. This suggests an important role for LDL cholesterol in developing OA joint pathology.

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WNT AND WISP1 EXPRESSION IN THE SYNOVIIUM INDUCES PRODUCTION OF CARTILAGE-DEGRADING METALLOPROTEINASES BY SYNOVIAL CELLS

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Purpose: Consequences of synovial activation, seen in many osteoarthritis (OA) patients, are largely unknown. The synovium mainly consists of fibroblasts, macrophages and we see monocytes in the early phases of our OA models. Previously, we found strongly increased expression of Wnt2b, Wnt16 and Wisp1, a downstream protein of canonical Wnt signaling, in knee joints in two murine OA models. Wnt signaling has been implicated in OA incidence through activation of the β-catenin-dependent canonical Wnt signaling pathway. In the present study, we investigated the potential of Wnt signaling to increase the expression of cartilage-degrading enzymes in the synovium.

Methods: Pathway analysis of microarray data from the synovium of a collagenase-induced OA mouse model was done using DAVID bio-informatics software. In vivo synovial overexpression of genes from the Wnt signaling pathway was achieved by intra-articular injection of adenoviral vectors. Human OA synovial tissue was collected from joint replacement surgery and either stimulated directly or used for outgrowth of OA fibroblasts. Monocytes were isolated from buffy coats from healthy donors and stimulated directly or after differentiation into M1 or M2 macrophages. Joint pathology was assessed by histology. Gene expression was analyzed by qPCR. Protein expression was measured in culture supernatants by Luminesix.

Results: Pathway analysis showed that Wnt signaling was enriched in the synovium during experimental OA. To determine the effects of Wnt signaling on the expression of cartilage-degrading enzymes in synovial tissue, we stimulated human OA synovial specimen with Wnt3a or its downstream protein WISP1. This resulted in increased expression of MMP3, MMP9 and MMP13, whereas expression of the MMP inhibitors TIMP1 and 3 was not altered. Next, we investigated which cell-type in the synovium might have caused the increased MMP expression. Stimulation of human synovial OA fibroblasts with Wnt3a or WISP1 increased the expression of both MMP3 and MMP13, whereas expression of MMP9 was not altered. In contrast, stimulation of both M1 and M2 macrophages with either Wnt3a or WISP1 did not result in increased expression of MMPs. In addition, we stimulated monocytes, which are present in the synovium in the early phases of our OA models. Stimulation of primary human monocytes with Wnt3a or WISP1 strongly increased the expression of MMP3, MMP9 and MMP13. Expression levels of TIMP1 and 3 were not altered. Next, we hypothesized that if increased Wnt signaling was present in OA synovial tissue and stimulation of synovial tissue with members of the Wnt signaling pathway increased the expression of MMPs, we should be able to decrease the expression of MMPs by blocking the Wnt signaling pathway. Inhibition of Wnt signaling by both FrzB and DKK-1, a specific inhibitor for canonical Wnt signaling, led to decreased expression of MMP3, MMP9 and MMP13 in human synovial specimen.

Finally, to determine if synovial overexpression of members of the Wnt signaling pathway leads to cartilage damage in vivo, we injected adenoviral vectors for Wnt5a, Wnt8a and Wnt16 into murine knee joints. These vectors specifically deliver Wnt proteins to synovial cells but do not penetrate into the cartilage, due to their size. Overexpression of Wnt8a and Wnt16 led to β-catenin accumulation, indicating signaling via the canonical Wnt signaling pathway, whereas Wnt5a overexpression did not. Seven days after overexpression, we found a significant induction of OA pathology after overexpression of Wnt8a and Wnt16, but not Wnt5a, at the medial meniscus and the medial tibial plateau, a preferential site for damage in experimental OA. Lesions were found in 92% n = 12 of the knee joints after Wnt8a overexpression compared to 17% n = 12 for the control

Figure 1. LDL cholesterol correlates with ectopic bone formation in the medial collateral ligament in experimental OA. Four representative microphotographs of the medial side of the knee joint, 36 days after induction of OA by intra-articular injection of collagenase. Both WT and ApoE deficient mice received a normal or a cholesterol-rich diet, starting 18 days before induction of OA. Safranin O - Fast Green staining.
814 INFRAPATELLAR FAT PAD INDUCES AN INFLAMMATORY AND CATABOLIC PHENOTYPE ON AUTOLOGOUS FIBROBLAST-LIKE SYNOVIOCYTICS FROM SEVERE KNEE OA PATIENTS

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Purpose: Infrapatellar fat pad (IFP) of the knee joint displays an inflammatory phenotype in osteoarthritis (OA). In addition to cartilage and bone tissues, OA pathophysiologic process involves synovial membrane, which is the seat of an heterogeneous inflammation. IFP location adjacent to the synovial membrane suggests that IFP could be involved in the induction of OA synovitis. We have investigated the response of fibroblast-like synoviocytes (FLS) to autologous IFP and subcutaneous adipose tissue (SCAT) from patients with severe knee OA.

Methods: IFP, SCAT and autologous synovial membrane closed to IFP were harvested during total knee replacement for severe OA from 28 patients. FLS from 14 patients were stimulated by autologous IFP- or SCAT-conditioned media and the gene expression and release of IL-6, IL-8, COX-2, TNF-z, PGE2, IL-1ß and IFN-y secretion by IFP and SCAT were quantified by ELISA. FLS were treated with PGE2 receptor antagonists to evaluate the contribution of PGE2 in the inflammatory response of FLS to IFP.

Results: IFP conditioned media induced the expression and the release of IL-6, IL-8 and PGE2, the expression of COX-2 and the expression and/or secretion of MPP-1, MPP-3 and MPP-9 by FLS. The stimulation was always stronger when FLS were stimulated with IFP as compared to SCAT conditioned media. Significantly higher amount of IL-6, IL-8, TNF-z and PGE2 was released from IFP to SCAT, especially PGE2 whose secretion was 70-fold higher by IFP (p < 0.0001). The rates of inflammatory mediators released by FLS were positively associated with PGE2 amounts produced by IFP. PGE2 receptor antagonists dose-dependently inhibited the release of IL-6, IL-8 and PGE2 by IFP-stimulated FLS.

Conclusion: Our results confirmed that IFP was a particular adipose tissue, with a higher inflammatory profile than SCAT from the same patient. We also brought out that IFP induced an inflammatory and catabolic phenotype on autologous FLS. Thus, in knee OA, IFP could contribute to the onset of inflammatory alterations within the synovial membrane especially through a high secretion of PGE2.

815 CROSS-SECTIONAL AND LONGITUDINAL ASSOCIATIONS BETWEEN KNEE JOINT EFFUSION AND OSTEOARTHRITIC STRUCTURAL CHANGES IN OLDER ADULTS

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Purpose: Multiple joint pathological changes such as synovial effusion, cartilage and subchondral bone lesions are involved in osteoarthritis (OA). The causal relationship between joint effusion and other knee structural changes was not clear. This study aimed to determine the cross-sectional and longitudinal associations between knee joint effusion at different compartments and knee osteoarticular changes in older adults.

Methods: A cohort of 976 randomly selected subjects from local community (mean 62 years, 50% female) was studied at baseline and 416 followed up 2.7 years later. Radiographic knee osteophyte and joint space narrowing (JSN) were measured and by the OARSI atlas. 72-weighted fat saturated magnetic resonance imaging (MRI) was utilized to assess knee effusion at 4 compartments: suprapatellar pouch, central, posterior, femoral recess, and subpopliteal recess. Cartilage volume, cartilage defects, and bone marrow lesions (BMLs) were measured using MRI at baseline and 2.7 years later. Multivariable generalized linear models with Poisson regression analyses or linear regression were used to estimate prevalence ratios (PR) or regression coefficient (b).

Results: Cross-sectionally, knee effusion at suprapatellar pouch was associated with lateral tibial (b = -76.4, p < 0.01) and patellar (b = -119.04, p < 0.01) cartilage volume, cartilage defect presence at any compartment (PR: 1.24, p < 0.01), any moderate to severe JSN (PR: 1.53, p < 0.01) and any osteophyte (PR: 1.53, p < 0.001). Effusion at central portion was associated with cartilage defect presence at any compartment (PR: 1.08, p = 0.05), BML presence at any compartment (PR: 1.19, p < 0.01) and lateral tibiofemoral osteophytes (PR: 1.69, p = 0.01). Effusion at posterior femoral recess was associated with patellar cartilage volume (b = 125.24, p < 0.01) and cartilage defect presence at any compartment (PR: 1.12, p < 0.01). Lastly, effusion at subpopliteal recess was associated with patellar cartilage volume (b = -79.35, p = 0.01), cartilage defect presence at any compartment (PR: 1.10, p < 0.01), BML presence at any compartment (PR: 1.13, p = 0.01), moderate to severe JSN (PR: 1.32, p < 0.01) and medial tibiofemoral osteophyte (PR: 1.31, p = 0.03). Longitudinally, suprapatellar pouch effusion was associated with change in medial tibial cartilage volume (b = -0.82%, p < 0.03), increases in cartilage defects at patellar and medial and lateral tibiofemoral compartments (RR: 1.24-1.32, p < 0.01), and an increase in BMLs at lateral tibiofemoral compartment (RR: 1.24, p = 0.04). Effusion at posterior femoral recess was associated with increases in medial tibiofemoral (RR: 1.26, p < 0.01) and patellar cartilage defects (RR: 1.31, p = 0.03) and an increase in any BMLs (RR: 1.39, p = 0.03). Effusion at subpopliteal recess was associated with change in patellar cartilage volume (b = -0.70%, p = 0.03), increases in medial tibiofemoral (RR: 1.31, p < 0.01) and patellar (RR: 1.15, p < 0.01) cartilage defects. In contrast, effusion at central portion was not significantly associated with changes in cartilage volume, cartilage defects or BMLs. All these analyses were performed after adjustment for after adjustment for age, gender, BMI, rheumatoid arthritis, and/or radiographic osteoarthritis (ROA).

Conclusions: Knee joint effusions are both cross-sectionally and longitudinally associated with knee osteoarticular structural changes suggesting a potential causal relationship. While suprapatellar pouch effusion is most consistently associated with knee structural changes, central portion effusion is not associated with changes in knee structures over time.