between N/R and I areas were identified. Many inflammatory mediators appear differentially expressed. The interferon alpha-inducible protein 6 (IFI6) was the most up-regulated. We also identified the hydroxysteroid (11-beta) dehydrogenase 1 (HSD11B1), the chemokine (C-X-C motif) ligand 1 (CXCL1) and the EBV-inducible G-protein coupled receptor 2 (EB2). The differential expression of interme-diates involved in angiogenesis pathway was also revealed between N/R and I areas. Among them, R-spondin-3 (RSP3), the secreted phosphoprotein 1 (SPP1) and aquaporin 9 (AQP9) were up-regulated whereas ADAMTS1 was down-regulated. Finally, in the Wnt signaling, R-spondin-3 up-regulated unlike dickkopf homolog 3 (DKK3) which was in turn down-regulated. We next performed a class comparison test between N/R and N/R-CS in one hand and between I and I-CS the other hand. 489 genes were identified as differentially expressed genes between N/R and N/R-CS conditions while 219 genes were identified between I and I-CS conditions. In this latter, our attention was focused on the down-regulated genes. Among them, we identified a number implicated in angiogenesis and cell migration pathways. Thus, the endothelial cell-specific molecule-1 (ESM1), the Transmembrane-4-L-six-family-1 (TM4SF1), the 5'-Ectonucleotide (NTSE) and the growth arrest-specific gene 6 (GAS6) were down-regulated by CS.

**Conclusions:** Our work demonstrates the differential gene expression profile between paired inflammatory and normal/reactive areas of synovial membrane as well as the modulatory effects of CS on gene expression in the inflammatory areas, especially regarding genes involved in both angiogenesis and cell migration.

555 INVESTIGATION OF POTENTIAL NEW TARGETS FOR THE DIAGNOSIS AND/OR THE TREATMENT OF OSTEOARTHRITIS

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**Purpose:** Synovial inflammation plays a key role in the pathophysiology process of osteoarthritis (OA). We have previously compared the gene expression pattern of synovial cells isolated from inflammatory (I) or normal/reactive (N/R) areas of a synovial membrane harvested from the same OA patient. We identified a large number of mediators belonging to key pathways involved in OA pathogenesis. The aim of this study was to validate different potential new targets for the diagnosis and/or the treatment OA.

**Methods:** Synovial cells (SC) were isolated from synovial specimens obtained from OA patients undergoing knee replacement. The inflammatory status of the synovial membrane was characterized according to macroscopic criteria. The biopsies from N/R and I areas were cultured separately for a period of 7 days. Microarray gene expression profiling between N/R and I areas was performed. The biological relevance of up- and down-regulated genes was analyzed with Ingenuity Pathways Analysis. Western blot and immunohistochemistry confirmed the identified genes most differentially expressed in the key pathways. The production of the triggering receptor expressed on myeloid cells-1 (TREM1), the alarmin S100 calcium binding protein A9 (S100A9), the wingless-type MMTV integration site family, member 5A (Wnt-5A) and the alarmin S100 calcium binding protein A9 (S100A9), the interferon alpha-inductible protein 6, Santa Cruz) and STAT-3 translocation (Stat3 inhibitor VIII, 5,15-DPP, Sigma-Aldrich) and STAT-3 inhibitor VIII, 5,15-DPP, Sigma-Aldrich) and Western blot. This result was also supported by the immunohistochemical analysis. In I area, the staining for STC1 was more intense in perivascular and sublining cells. A significant increase of STC1 production was STC1. A significant increase of STC1 production was observed in I areas compared to N/R areas by Western blot. This result was also supported by the immunohistochemical analysis. In I area, the staining for STC1 was more intense in perivascular and sublining cells. This investigation has to be further pursued.

556 CHONDROITIN SULFATE REGULATES TWO ANTI-ANGIOGENIC FACTORS IN HUMAN OSTEOARTHRITIC SYNOVIAL FIBROBLASTS

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**Purpose:** The symptomatic slow acting drug for osteoarthritis (SYSOA), chondroitin sulfate, has attracted much interest as a biological agent for its use in the effective relief of disease symptoms. This is likely due to its capacity for reducing joint swelling and effusion which may be explained by its anti-inflammatory effects. However, the factors involved in this latter effect are yet to be fully identified. Angiogenesis is an important event of synovial inflammation and it is suggested to contribute to the joint symptoms in OA. We thus elected to determine the effect of chondroitin sulfate on the levels of two well-known anti-angiogenic factors previously demonstrated to be involved in OA: the vascular endothelial growth inhibitor (VEGI) and thrombospondin-1 (TSP-1). Furthermore, we explored the signaling pathways by which chondroitin sulfate exerts its effects on these factors.

**Methods:** Human OA synovial fibroblasts were obtained from sequential enzymatic digestion. Cells were pre-incubated for 18 hours with chondroitin sulfate (200 μg/ml; CS-6-Active®, Bioébrica, Spain) and then incubated with IL-1β (10 and 100 pg/ml) in the absence or presence of chondroitin sulfate for increasing periods of time. Cells were processed for protein expression determination using qualitative PCR, protein production using ELISAs, and for signaling pathways using Western blotting.

**Results:** Both VEGI and TSP-1 expression were dose-dependently decreased by IL-1β at both concentrations tested. Chondroitin sulfate significantly increased the expression and synthesis levels of the two anti-angiogenic factors VEGI and TSP-1 under basal conditions as well as under IL-1β treatment. The signaling pathways through which chondroitin sulfate increased the levels of VEGI and TSP-1 in the presence of IL-1β were further investigated by measuring the phosphorylated levels of three MAP kinases, namely ERK1/2, p38, and JNK, and other cascades including NF-κB, Akt, and STAT-3. Data revealed that the effects occurred through two main pathways, Akt and STAT-3. Additional experiments with specific inhibitors of Akt phosphorylation (SH-6, Santa Cruz) and STAT-3 translocation (Stat3 inhibitor VIII, 5,15-DPP, Santa Cruz) confirmed these data.

**Conclusions:** This study showed that chondroitin sulfate increased the levels of two important anti-angiogenic factors, which could provide a possible new mechanism of action of this SYSOA. These findings may explain, in part, how chondroitin sulfate reduces the severity of synovitis in knee OA. In addition, these data bring to light that anti-angiogenic factors could be targeted as a specific therapeutic approach in disease modifying OA drug development.
elucidate which genes downstream of TGF-β are elevated in synovial fibrosis in OA. To this end we measured 37 genes involved in extracellular matrix turnover in TGF-β stimulated human synovial OA fibroblasts (hSOAF) in the synovium of mice with TGF-β-induced synovial fibrosis. Importantly, these genes were also measured in human end stage OA synovium. This study will unravel which TGF-β-responsive genes are elevated in OA-related fibrosis and may reveal potential targets for therapeutic intervention.

Methods: Synovial fibrosis was induced in C57Bl/6 mice (n = 5 per group) by intra-articular (i.a) injection of an adenovirus encoding active TGF-β (Ad-TGF-β) and compared to control virus. At day 21 after injection the mice were sacrificed. We induced OA by i.a injection of bacterial collagenase into the right knee joint of C57Bl/6 mice (n = 6 per group). Mice were sacrificed 42 days after injection. mRNA was isolated from synovium for microarray and Q-PCR analysis. We isolated hSOAF out of synovium obtained from OA patients (n=7) undergoing knee joint arthroplasty (after informed consent). The hSOAF were transduced with Ad-TGF-β and control virus. Furthermore mRNA was isolated from synovial tissue punches obtained from knee joints of patients with end stage OA and from controls for microarray analysis.

Results: In hSOAF transduced with Ad-TGF-β, 21 out of 37 fibrosis-related genes were upregulated over 2-fold compared to control virus (fig 1). In the synovium from mice with collagenase-induced OA and from human with end stage OA, we found 20 fibrosis related genes, from which 12 overlapping, that were more than 2-fold upregulated. In general there is increased gene expression of procollagens and cross linking genes. Comparing the three different conditions, revealed an overlap of PLOD2, P4HA3, LOX, COL1A1, COL5A1, TIMP1, and MRC2 (fig 1). We also found that these genes were highly upregulated in Ad-TGF-β induced fibrosis: PLOD2 (13-fold), P4HA3 (60-fold), LOX (9-fold), COL1A1 (18-fold), COL5A1 (6-fold), TIMP1 (10-fold), and MRC2 (11-fold).

Fig 1. Fibrotic genes that are at least 2-fold upregulated in TGF-β stimulated OA fibroblasts, synovium of mice with collagenase-induced OA and in synovium obtained from patients with end stage OA.

Conclusions: We found that PLOD2, P4HA3, LOX, COL1A1, COL5A1, TIMP1, and MRC2 more than 2-fold upregulated in TGF-β stimulated OA fibroblasts, in collagenase-induced OA, in TGF-β-induced fibrosis as well as in human end stage OA suggesting key roles in OA-related fibrosis. For therapeutic purposes, most of these genes will be poor targets, due to their crucial functions in the joint. Fortunately there is an exception: PLOD2, encoding lysyl hydroxylase 2b (LH2b), is an attractive candidate. LH2b increases pyridinoline cross-link formation during collagen synthesis, making collagen less susceptible to degradation. This might lead to collagen accumulation and result in thickening and stiffening of the synovial membrane. We propose that PLOD2 plays a key role in OA-related synovial fibrosis as it is elevated in collagenase-induced OA, TGF-β-induced synovial fibrosis and human end stage OA. This makes PLOD2 an interesting target to block in order to inhibit/prevent fibrosis, which would be a major step forward in the treatment of OA.

558 SYNOVIAL FLUID LIPID COMPOSITION OF NORMAL HORSE METACARPOPHALANGEAL JOINTS
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Purpose: Normal synovial fluid (SF) is a biological lubricant and contributes to the transport of nutrients to the joint cartilage and to the joint cleaning and defense. Although lipids are not present in a large quantity in SF they participate to both normal SF homeostasis and biomechanic. During joint diseases, SF lipids contribute to the inflammatory and apoptotic processes and their role in the SF lubricating properties is impaired. The review of the literature shows gap and discrepancies in SF lipids study. This prompts the need to accurately define normal SF lipids as they are potential joint disease biomarkers and therapeutic targets. We thus propose to study the lipidomic of SF in the horse who is both a target species for joint diseases and an experimental model of osteoarthritis. We present preliminary data on the qualitative analysis of SF lipids in healthy horse fetlocks.

Methods: Synoviocentesis of the right metacarpophalangeal joint was performed in six sound horses (5 geldings, 1 mare, mean weight 536+/- 50kg, and aged from 10 to 17 yo). SF volume, gross aspect, viscosity and index of refraction were noted. The method of Folch was used for lipids extraction and purification. The protein content was determined according to the Bradford method. The separation of lipid classes was performed by solid phase extraction (SPE) on aminopropyl cartridges. Individual lipids of the different fractions were identified by 1 or 2 dimensions thin layer chromatography (TLC) after being normalized to total protein content.

Results: SF was yellowish, viscous, adhesive and exhibited thixotropy. Mean volume was 2.9+/-1.6 ml, SF thread test was 2.3 +/-1.2 cm and index of refraction was 1.022 +/- 0.005. Mean total lipid concentration was 3.5 +/-2.3 mg/ml. Mean total protein concentration was 4.92+/-2.7 mg/ml. 1D-TLC of neutral lipids showed that they are mainly composed of cholesterol, triacylglycerol, and cholesterol esters (Fig 1A). Free ceramides were also expressed in normal SF as shown in Fig 1B. Interestingly, two main spots of ceramides (corresponding to type III and IV) were detected, suggesting that particular molecular species of ceramides with high hydroxylation degree occurs in this SF. 2D-TLC also highlighted the expression of neutral glycosphingolipids within SF, although at low extent (Fig 1C). 2D-TLC of polar lipids demonstrated that choline-containing phospholipids (phosphatidylcholine (PC), sphingomyeline and Lysophosphatidylcholine (LPC)) are the most abundant, with traces of phosphatidylyethanolamine (PE) and phosphatidinositosils (PI) (Fig 1D).

Conclusions: We determined the gross composition of lipids within the SF of normal horse fetlock. Based on these results, we will determine the full molecular composition of these lipid classes both in normal SF and in pathologic conditions. In particular, the study of lipid homeostasis would allow for the development of new biomarkers and could be used to guide specific therapy in the management of joint disease.