a novel inhibitor of matriptase shows good selectivity for matriptase compared to other trypsin-like serine proteases.

**Conclusions:** Building on evidence that matriptase acts as a novel initiator of cartilage collagen destruction, these new data show that it can also potently degrade cartilage proteoglycan by induction of MMPs and ADAMTS4. We have demonstrated that this breakdown is largely due to the activity of MMPs. The matriptase inhibitor appears to be potent and selective towards matriptase and represents a valid start point for drug development in OA.

**225**

**GLYCAN MAPPING AND GALECTIN-1 EXPRESSION IN OSTEOARTHRITIC CARTILAGE**

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**Purpose:** The glycosylation of cells and tissues is a complex process that contributes to the adhesion capacity of cells and potentially affects cellular signal transduction or the onset of apoptosis. In this context, the glycan contribution to the adhesion capacity of cells and potentially affects cellular glycosylation of cells and tissues is a complex process that contributes to the adhesion capacity of cells and potentially affects cellular signal transduction or the onset of apoptosis. In this context, the glycan-encoded information may be translated into a biological effect by endogenous lectins such as galectins. Despite the significance of glycoproteins for the maintainance of cartilage function, the potential implications of the chondrocyte glycophenotype in the pathophysiology of osteoarthritis (OA) remain largely unknown. The present study aimed to assess the glycan profile and the expression of Galectin-1 in human OA cartilage and to determine the effect of Galectin-1 on primary chondrocytes in vitro.

**Methods:** Cartilage specimens were collected from OA patients undergoing knee replacement surgery (n=10) prior to processing for immunohistochemistry. Following staining with safranin-O, the grade of cartilage degeneration was defined in 90 histological sections using the Mankin degeneration score. Using a panel of 11 plant lectins, common constituents of natural glyans, serving as ligands for endogenous lectins (galectins), were attributed to the tissue sections. Histochemical analysis of Galectin-1 binding sites was accomplished using biotinylated human Galectin-1. The number of Galectin-1 positive chondrocytes was evaluated in cartilage specimens as a function of the degeneration score. The biological effect of Galectin-1 on isolated primary human chondrocytes was determined using RT-qPCR and ELISA assays.

**Results:** The intensity of plant lectin staining was subject to the grade of cartilage degeneration. Cartilage tissues with a high Mankin score were characterized by strong plant lectin staining indicating the expression of functional glycan epitopes in degenerated cartilage. Moreover, binding sites for Galectin-1 were detected in OA cartilage. Importantly, a significant correlation was revealed between the number of Galectin-1 positive chondrocytes and the degeneration score (r=0.71). RT-qPCR and ELISA assays demonstrated that Galectin-1 strongly upregulated Interleukin-1B as well as Matrix metalloproteinase-3 and -13 mRNA and protein levels in cultured chondrocytes.

**Conclusions:** Viewing OA from a glycobiological perspective, we found that the expression of glycan epitopes was significantly more pronounced in highly degenerated OA cartilage compared to intact cartilage. Of note, the expression of Galectin-1 by chondrocytes correlated with the cartilage degeneration score, indicating the potential translation of the sugar code into biological effects. In this context, we demonstrated Galectin-1 binding sites in OA cartilage and showed that Galectin-1 induced catabolic processes in chondrocytes in vitro. In summary, this study suggests a role for altered glycan profiles and Galectin-1 expression in OA disease progression.

**226**

**CARTILAGE DAMAGE IN VIVO AND IN VITRO REGULATES MOLECULES THAT DRIVE PAIN IN MURINE OSTEOARTHRITIS**

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**Purpose:** Pain is the main symptom of osteoarthritis (OA), but it is currently unknown which tissues give rise to pain and what drives it in OA.

Our previous studies in painful behaviour in mice following surgical induction of OA revealed that mice develop pain at around 8 weeks post-surgery. At this time there is moderate cartilage damage, but little synovitis in the joint. The aim of this study was to identify which pain pathways are activated in painful OA joints, in which tissues are these pathways activated, and what is driving them.

**Methods:** Partial meniscectomy or sham operation was performed in 10 week old female C57Bl6 mice. Pain assessments were performed weekly. RNA was isolated from whole knee joints, or from micro-dissected tissues (articular cartilage, meniscus and tibial epiphysis). For cartilage injury responses in vitro, RNA was isolated from hip epipheyes of 5 week old mice, at 0 and 4h post-explantation. RT-PCR was performed using Taqman Low Density Arrays for 67 genes that are involved in inflammation and pain.

**Results:** Pain developed 8 weeks after partial meniscectomy. Bradykinin receptors (BDKR1 and 2), protachykinin (TAC1), tachykinin receptor 1 (TACR1), nerve growth factor (NGF), neuropeptide Y (NPY) and CCL21 were the only genes upregulated in the whole joint compared to sham operated joints from the panel of 67 genes. When we examined the microdissected tissues we found that the same genes, apart from BDKR2B and NPY, were upregulated in cartilage. BDKR12 was upregulated in the epiphysis. NPY was not regulated in any of the microdissected tissues. To establish whether cartilage injury per se could induce these same genes, we looked at the regulation of pain related genes in explanted murine hips. NGF, BDKR1B, TAC1 and NPY were strongly regulated upon cartilage injury in vitro.

**Conclusions:** This study has revealed a number of important observations:

- A select group of pain mediators and receptors are regulated in the joint when meniscectomised mice develop pain.
- Expression of these mediators and receptors is occurring in a non-inflamed joint, and is not associated with expression of other inflammatory molecules.
- The majority of these pain mediators and receptors are expressed in the damaged cartilage in vivo and regulated by cartilage injury in vitro.

**227**

**THE MOLECULAR RESPONSE TO ACUTE JOINT DESTABILISATION IS ALTERED IN FEMALE COMPARED TO MALE MICE.**

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**Purpose:** It is well established that gender is an independent risk factor for the development of osteoarthritis (OA). Human epidemiological studies have highlighted the increased incidence of disease in women after the age of 50 years. This postmenopausal increase in OA has been attributed to changes in sex hormones, and has led to the suggestion that estrogen has chondro-protective effects. This is supported by the observation that the disease severity of surgically induced OA in female pre-menopausal mice is significantly lower than in males, and that in female mice it is increased following oophorectomy. The effect of sex hormones on OA severity could be at the level of the joint tissues themselves, or could be indirect by affecting other factors such as levels of physical activity. The aim of this project was to investigate the mechanisms by which gender affects severity of OA.

**Methods:** OA was induced in 10 week old male and female C57Bl6 mice by destabilisation of the medial meniscus (DMM). OA was assessed by histological assessment of the joints at 4, 8 and 12 weeks post DMM or sham surgery. Activity levels in mice were assessed using LABORAS (Laboratory Animal Behaviour Observation Registration and Analysis System) which is able to distinguish different types of activity (running, eating, climbing, resting). Pain assessments were performed using the Linton incapacitance tester (which measures differential weight borne in the operated compared to non-operated joints). RNA was extracted from mouse knee joints for RT-PCR analysis 6h following surgery to determine whether gender influences the molecular response to acute joint destabilisation.

**Results:** Histological analysis confirmed previous studies demonstrating an increase in severity of OA in male compared to female mice at 4, 8 and