minor (Gal-8C) activity, indicating a requirement for bivalency of Gal-8. Genome-wide microarray in combination with bioinformatics and molecular analyses revealed that Gal-8S reprograms the gene expression pattern of OA chondrocytes towards a pro-degradative/inflammatory signature that involves the activation of NF-kB (Fig. 1D). Bioinformatics further revealed a high level of congruence between Gal-8S, Gal-1 and Gal-3 regarding their imparted gene signatures and activated pathways, but also indicated evidence for complementary activities. Mimicking the physiological setting, the addition of subsaturating Gal-8S concentrations to chondrocytes treated with mixtures of Gal-1 and -3 significantly increased IL1B and MMP13 levels, suggesting functional cooperation between the three galectins (Fig. 1E).

Conclusions: This report establishes the functional significance of Gal-8 as a broad-spectrum upstream effector in OA. Gal-8 induced a pro-degradative/inflammatory gene signature, largely under control of NF-kB signaling. Of note, our work discovered a functional cooperation between Gal-8, -3 and -1, three galectins that largely differ in molecular architecture, providing an illustrative precedent for future studies on the clinical relevance of cross-talk among galectins.

23
INHIBITION OF LRP1 SHEDDING REVERSES CARTILAGE DEGRADATION IN OSTEOARTHRITIS

K. Yamamoto †, C. Scavenius ‡, S. Santamaria §, K.A. Botkjaer ‖, J. Dudhia ¶, L. Troeberg #, Y. Itoh #, G. Murphy ‖, J.J. Enghild ‡, H. Nagase #. † *Inst. of Ageing and Chronic Disease, Univ. of Liverpool, Liverpool, United Kingdom;* ‡ *Dept. of Molecular Biology and Genetics, Aarhus Univ., Aarhus, Denmark;* § *Ctr. for Haematology, Imperial Coll. London, London, United Kingdom;* ‖ *Cancer Res. UK Cambridge Inst., Univ. of Cambridge, Cambridge, United Kingdom;* ¶ *Dept. of Clinical Sci. and Services, Royal Vet. Coll., Herts, United Kingdom;* # *Kennedy Inst. of Rheumatology, Univ. of Oxford, Oxford, United Kingdom*

Purpose: There is no clear correlation between mRNA levels of ADAMTS5 and progression of osteoarthritis (OA). We have found that ADAMTS5 is constitutively produced in healthy human cartilage, but are rapidly taken up and degraded intracellularly by the chondrocytes via the low-density lipoprotein receptor-related protein 1 (LRP1). Other proteins that are endocytosed by LRP1 include ADAMTS4, MMP13 and TIMP3, indicating that LRP1 is a key extracellular regulator of the cartilage matrix-degrading systems. However, this endocytic system is impaired in OA cartilage due to increased ectodomain 'shedding' of LRP1. The aim of this study is to identify the LRP1 sheddase(s) in human cartilage, and to test whether inhibition of LRP1 shedding prevents the cartilage matrix degradation in OA.

Methods: Cell-associated LRP1 and soluble LRP1 (sLRP1) released from human cartilage explants and chondrocytes were measured by Western blot analysis. LRP1 sheddases were identified by protease inhibitor profiling and gene silencing with siRNAs. Specific monoclonal antibodies were used to selectively inhibit the sheddases. Degradation of aggrecan and collagen in human OA cartilage was measured by Western blot analysis using an aggrecan neo-epitope antibody and hydroxyproline assay, respectively.

Results: Membrane-bound LRP1 was reduced in OA compared with normal cartilage, which was accompanied by an increased release of shed sLRP1 into the medium. sLRP1 bound to ADAMTS5 and MMP13 and prevented their endocytosis without interfering with their proteolytic activities. LRP1 shedding was partially inhibited by knockdown of MMP14 or ADAM17. Double knockdown of MMP-14 and ADAM-17 exhibited an additive effect. Combination of inhibitory antibodies against ADAM17 and MMP14 blocked LRP1 shedding in OA cartilage and increased the endocytic capacity of OA chondrocytes close to that of normal chondrocytes. Remarkably, combined antibody treatment reduced the degradation of aggrecan and collagen in OA cartilage.

Conclusions: Two membrane-bound metalloproteinases, ADAM17 and MMP14, were identified as the LRP1 sheddases in cartilage. Inhibition of their activities reverses cartilage matrix degradation in OA cartilage. Local inhibition of LRP1 shedding is a unique option for OA therapy, as the recovery of the lost endocytic function of chondrocytes would help to maintain cartilage homeostasis. Furthermore, unbalanced tissue homeostasis due to dysfunction of the endocytic system may become the causes of other human diseases such as cancer, atherosclerosis and neurodegenerative diseases as LRP1 is widely expressed in many tissues.

24
GENOMIC AND PROTEOMIC BIOMARKER SCREENING SHOWS AUTOPHAGY DEFECTS IN BLOOD AND CHONDROCYTES IN OSTEOARTHRITIC PATIENTS

I. Lorenzo-Gomez †, N. Oreiro †‡, S. Relaño †, F.J. Blanco †, B. Carames †. † *INIBIC, A Coruña, Spain;* ‡ *CHUAC, A Coruña, Spain*

Purpose: In osteoarthritis (OA), defects in cellular homeostasis, and in particular in autophagy, are evident and precede joint damage. In this sense, we have shown that there is a decrease in autophagy markers in chondrocytes and cartilage from OA patients, and pharmacological activation of autophagy protects against joint damage. These data suggest that joint damage presented by patients with OA could be due to a failure of autophagy, inducing an abnormal accumulation of cellular products related to disease. These observations represent a unique opportunity to identify and validate biomarkers associated with autophagy that could facilitate the development of therapeutic strategies to prevent OA progression.

Methods: A comparative analysis of 86 autophagy genes was performed in blood from control and knee OA patients. Control patients (Age: 61,60 ± 1,57 years; BMI: 25,54 ± 0,74; Sex: Females; N = 10) and Knee OA patients (Age: 64,60 ± 1,59 years; BMI: 30,76 ± 0,94; Sex: Females; N = 10, OA grade III–IV) were profiled using a human autophagy PCR array (PrimePCR autophagy human panel, BioRad) and analysed using the PrimePCR analysis software, Biorad. In addition, we performed a quantitative proteomic analysis of Atg5 knockdown OA primary human chondrocytes by using iTRAQ (isobaric tags for relative and absolute quantitation) labeling coupled with on-line 2D LC/MS/MS. Protein identification and quantification were performed using Protein Pilot Software v 4.0. Each MS/MS spectrum was searched in the Uniprot/Swissprot database for *Homo sapiens*.

Results: The results showed that in blood from knee OA patients 16 autophagy-related genes were significantly down-regulated compared to blood from control patients. No significant up-regulation was observed in blood from Knee OA patients, however a trend-toward up-regulation was detected in several genes involved in the mTOR signaling pathway. Importantly, 5 key autophagy-related genes, such as ULK1, ATG16L2, ATG12, ATG7 and MAP1LC3B involved in initiating autophagy, phagophore extension and autophagosome formation were downregulated in blood from knee OA patients compared to control patients ($p<0.05$). In addition, chaperone-mediated autophagy genes, HSP90AA1 and HSPA8 were also significantly downregulated ($p<0.05$) in blood from knee OA patients. Other regulators of autophagy and apoptosis, such as BNIP3 and Bcl-2 were also significantly downregulated in OA patients ($p<0.01$). Proteomic screening of human OA chondrocytes with defective autophagy by siAtg5, show a significant reduction of Heat shock protein HSP 90-alpha (HSP90AA1) ($p<0.05$), a chaperone-mediated autophagy, suggesting that reduced autophagy could be a biomarker for OA progression and development.

Conclusions: Molecular analysis of autophagy at a systemic level in OA would facilitate the identification of biomarkers of early diagnosis and progression of OA.

25
INTEGRIN α10β1-SELECTED MESENCHYMAL STEM CELLS MITIGATE PROGRESSION OF POSTTRAUMATIC OSTEOARTHRITIS IN AN EQUINE ARTICULAR IMPACT MODEL

M.L. Delco †, J.F. Talts ‡, S.L. Powndner §, M. Koff §, E. Lundgren-Åkerlund ‡, L.A. Fortier †. † *Cornell Univ., Ithaca, NY, USA;* ‡ *Xintela AB, Lund, Sweden;* § *Hosp. for Special Surgery, New York, NY, USA*

Purpose: Mesenchymal stem cell (MSC)-based therapies have been investigated for the treatment of osteoarthritis (OA), and can reduce clinical symptoms of OA and improve cartilage morphology. Early intervention after articular trauma has the potential to limit the progression of focal lesions and prevent ongoing cartilage degeneration by modulating the joint environment and/or contributing to repair. Integrin α10β1 is the collagen type II binding receptor on chondrocytes, and MSCs that express α10β1 have improved chondrogenic potential. The purpose of this study was to investigate the safety and efficacy of integrin α10β1-selected mesenchymal stem cells (α10β1+MSCs) to prevent the development of posttraumatic osteoarthritis in an equine model of impact-induced ankle arthritis.