OP0259

ZCCHC5, A LTR RETROTRANSPONSON-DERIVED NEOFUNCTIONALIZED GENE, IS ESSENTIAL FOR THE TRANSCRIPTIONAL ACTIVITY OF SOX9 AND THE EXPRESSION OF COL2A1 IN CHONDROCYTES AND IS DOWNREGULATED IN OA CARTILAGE

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Background: Retrotransposon-derived DNA sequences occupy approximately 40% of the mammalian genome, compared with only 1.5% of protein coding genes, and are a source of variation in the genome. LTR-derived gene *ZCCHC5* (*Mart3*) is a member of *gag*-related retrotransposon family that has lost the ability to retrotranspose. *ZCCHC5* gene encode a protein of approximately 53 Kd and contains a nucleic acid binding domain (CX₂CX₄HX₄C), a *gag*-like region within the intact ORF and a homeobox associated leucine zipper motif indicating that this gene may have acquired new function(s) in the cell. Expression and function of *ZCCHC5* in degenerative joint diseases such as OA or other diseases has not been explored.

Objectives: The aim of this study was to investigate whether *ZCCHC5* is expressed in OA cartilage and chondrocytes and whether it is involved in the regulation of catabolic and/or anabolic factors in chondrocytes and its modulation under pathological conditions.

Methods: Chondrocytes were derived by the enzymatic digestion of human OA and normal C57BL6 mouse cartilage. Total RNA was prepared using Trizol and was made DNA-free using on-column digestion. mRNA expression was quantified using TaqMan assays. Protein expression was determined by immunohistochemistry (IHC) and Western blotting (WB) with validated antibodies. siRNA mediated depletion or plasmid mediated overexpression of *ZCCHC5* gene was used to study its role in chondrocyte function under pathological conditions. Luciferase reporter vectors were used to study promoter activity in human chondrocytes.

Results: TaqMan, WB and IHC analyses revealed that the expression of *ZCCHC5* was very low in the damaged OA cartilage compared to the levels detected in normal cartilage and the unaffected cartilage of the same patient. Stimulation of human and mouse chondrocytes with IL-1 β significantly decreased the expression of *ZCCHC5* ($p < 0.05$) which correlated with the inhibition of COL2A1 expression and upregulation of MMP-13. Overexpression of *ZCCHC5* upregulated the COL2A1 mRNA and protein levels which were not suppressed by stimulation with IL-1 β . Importantly, in these cells expression of MMP-13 was low and remained suppressed under pathological conditions. In contrast siRNA mediated depletion of *ZCCHC5* expression in chondrocytes abrogated the protective effect on COL2A1 expression and increased the level of MMP-13 mRNA and protein upon treatment with IL-1 β . Additionally, depletion of *ZCCHC5* eliminated the SOX9 activity and COL2A1 promoter activation in human chondrocytes but this was reversed by reintroduction of *ZCCHC5* expression indicating that *ZCCHC5* plays an important role in SOX9 activity and COL2A1 transcription.

Conclusions: Our data for the first time demonstrate that a LTR retrotransposon-derived neogene *ZCCHC5* plays a key role in maintaining the differentiated functions of adult articular chondrocytes. Its repression in OA may contribute to cartilage deterioration by blocking chondrocyte anabolic functions and by enhancing expression of catabolic factors. Taken together, our data revealed a previously unidentified role of *ZCCHC5* in a disease pathogenesis and uncovered a potential therapeutic approach to limit/reverse cartilage damage in OA.

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OP0260

TARGETING NEUTROPHIL MICROVESICLES TO DAMAGED CARTILAGE USING ANTIBODIES TO POST-TRANSLATIONALLY MODIFIED COLLAGEN II

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Background: Microvesicles (MV) are extracellular vesicles released from the plasma membrane of cells. MV derived from neutrophils (PMN) have been shown to penetrate cartilage and exert chondro-protective effects in inflammatory arthritis 1-2.

Collagen type II (CII) is the most abundant protein found in cartilage. Inflammation in the joint results in post-translational modification of collagen II (CII) by reactive oxygen species (ROS). We have produced a single chain variable fragment (scFv) antibody specific to CII modified by ROS, namely anti-ROS-CII³. We hypothesise that loading anti-ROS-CII upon MV will localise them to the arthritic cartilage.

Objectives: The main objectives of this study were: i) Load anti-ROS-CII upon MV using aqueous energy dissemination; ii) Assess incorporation of anti-ROS-CII upon MV *in vitro* and iii) Validate the localisation and therapeutic potential of the enriched microvesicles in an *in vivo* model of arthritis.

Methods: Anti-ROS-CII were produced in house. Human PMN MV from healthy donors were isolated as previously described⁴. Anti-ROS-CII were intercalated upon MV using 100 ug 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine by aqueous energy dissemination, as previously described⁴⁻⁵. Incorporation was assessed by Imagestream^X (IS^X), ELISA, NanoSight and immunofluorescence.

In vivo experiments were performed in an Antigen Induced Arthritis (AIA) mouse model. Localisation was assessed by IVIS Imaging following intravenous treatment of enriched MV. *Ex vivo* IVIS imaging was performed to confirm *in vivo* data. *In vivo* experiments were also conducted using MV enriched with anti-ROS-CII fused to vIL-10 and anti-mTNF therapies. Clinical scores were assessed throughout. Knee joints were harvested and snap frozen to detect the presence of enriched MV in the arthritic cartilage by confocal microscopy, and for qPCR analysis.

Results: Incorporation of anti-ROS-CII upon MV was observed by Imagestream. Antibodies present on the MV retained their binding capabilities, as shown by ELISA. Strong microvesicle fluorescence was detected in the arthritic cartilage of mouse tissue sections when using anti-ROS-CII enriched MV as a primary incubation.

In vivo studies exhibited the ability of the enriched MV to localise specifically in the arthritic joint. *Ex vivo* IVIS imaging confirmed *in vivo* results. *Ex vivo* knee cryosections showed the presence of enriched MV specifically within the arthritic cartilage.

In vivo treatment studies using anti-ROS-CII fused to anti-inflammatory therapeutics led to a significant reduction in knee swelling.

Conclusions: In this study we have demonstrated the ability to use aqueous energy dissemination to successfully enrich MV with antibodies. These enriched MV are able to localise in the arthritic joint and deliver anti-inflammatory therapeutics.

Overall, this study demonstrates the attainability of targeting a biological scaffold to the arthritic joint. The potential of co-delivering MV alongside anti-inflammatory therapeutics is paramount to simultaneously protect cartilage and reduce inflammation.

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OP0261

RETINOIC ACID IS REGULATED BY CARTILAGE INJURY AND IS ANTI-INFLAMMATORY IN HAND OSTEOARTHRITIS

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Background: A Genome-Wide Association Study (GWAS) in hand OA has identified the association of hypomorphic variants within *ALDH1A2* and severe hand OA (Styrkarsdottir U, et al. 2014). This gene encodes the enzyme catalysing the production of all-trans retinoic acid (atRA). atRA has an essential role in embryonic limb development, but its role in adult cartilage and in OA remains unclear. Previous work from our lab has shown that cartilage injury activates inflammatory signalling and regulates the expression of inflammatory genes.