and PRO-C2), aggrecanase degraded aggrecan (AGNxl) and MMP degraded aggrecan (exFFGV). C1M and C3M are synovial membrane biomarkers and C2M, AGNxl and exFFGV are cartilage biomarkers.

Results: Explants were viable throughout the experiments, albeit the bSME lost some viability with time. bSME treated with O+T showed increased C1M and C3M (>400% and >200%) from day 10, compared to w/o, whereas in bCC O+T increased C1M from day 21 and C3M from day 14 (>800%, >1900%). O+T treatment increased C2M was increased from day 21 (>400% and >1000%) in both BEX and bCC. The release was blocked by the generic MMP inhibitor GM6001 which also decreased the C1M and C3M compared to w/o. O+T treatment increased AGNxl at day 7 and 10 (>600%) and exFFGV from day 21 (>650%) in both BEX and bCC. In bSME, TGF- β 1 continuously and dose-dependently increased P1NP from day 7 compared to w/o (250%). O+T pre-treatment for 10 days followed by TGF- β 1 stimulation increased P1NP after 7 days of TNF- β 1 treatment (150%, figure). IGF-1 did not affect the P1NP level at any time point in bSME. In bCC both TGF- β 1 (dose-dependently) and IGF-1 sustained the PRO-C2 level at the level of baseline throughout the study periods (figure), whereas O+T decreased PRO-C2 compared to with w/o. The PRO-C2 level in BEX with TGF- β 1 was unaltered compared to w/o.

Conclusions: We here show that bovine synovium can be anabolic and catabolic stimulated, both alone and in co-culture with cartilage. Anabolic stimulation was achieved with TGF- β 1 in both bSME and bCC, while IGF-1 only showed an anabolic effect in the bCC. Previous human explant models using human tissue have lacked the anabolic capacity. These translational explant models may be applied in the early development of anabolic drugs for cartilage degenerative diseases.

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FRI0004 14-3-3ETA AS A NOVEL INVADOSOME REGULATORY MOLECULE IN RHEUMATOID ARTHRITIS

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Background: Progressive cartilage destruction, mostly mediated by invasive fibroblast-like synoviocytes (FLS), is a central feature in the pathogenesis of rheumatoid arthritis (RA). We have reported that the ability of arthritic FLS to degrade the extracellular matrix depends on the formation of actin-rich plasma membrane invadosomal structures detected in cells strategically located at the cartilage-synovial membrane interface. Interference with the formation of invadosomes in RA FLS impeded matrix degradation in vitro and cartilage degradation in a model of collagen-induced arthritis, suggesting that invadosomes are important physiological structures involved in cartilage destruction.

The chaperonin molecule 14-3-3 η has been detected in the joints of patients with early and established RA and that its concentration in both serum and synovial fluid correlated with elevated expression of extracellular matrix (ECM) degrading enzymes and erosive damage. Extracellular 14-3-3 η has therefore been proposed to be a novel biomarker for joint damage and a potential drug target for the personalized treatment of connective tissue-associated diseases but the direct relationship between 14-3-3 η and joint damage remains a key area of research.

Objectives: To evaluate the role of 14-3-3 η in the ability of synoviocytes to degrade the ECM.

Methods: mRNA from primary synoviocytes of healthy individuals (N=3) and RA patients (N=5) was extracted and the relative level of 14-3-3 isoforms and MMP

gene expression was determined by qPCR. The ability of the synovial cell lines to degrade ECM was assessed by in situ invadosome assays using fluorescent cross-linked gelatin. Confocal microscopy was used to determine the cellular localization of 14-3-3 η .

Results: We found a significant increase in 14-3-3 η , MMP1 and MMP3 mRNA levels in synoviocytes from rheumatoid arthritis patients compared to cells from non-arthritis individuals. A strong correlation between 14-3-3 η expression levels and the ability of synoviocyte cell lines to form invadosomes was observed ($r^2=0.8299$). Knockdown of 14-3-3 η decreased the ability of arthritic synoviocytes to form invadosomes indicating a role of 14-3-3 η in extracellular matrix degrading ability. Confocal microscopy revealed that 14-3-3 η staining was mostly found as small punctated structures in the cytoplasm and at the cell periphery of arthritic synoviocytes where they colocalized with leading edge F-actin and discrete patches of the exocyst component, Exo70.

Conclusions: The finding of the role of 14-3-3 η in invadosome formation points to a previously unappreciated facet of how 14-3-3 η influences joint ECM remodelling and reinforces its role as a marker of RA progression and joint damage. How 14-3-3 η is involved in the regulation of MMP production/secretion and the possible role it plays in remodelling of actin-rich subcellular structures is the subject of ongoing studies.

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FRI0005 TARGETING CARTILAGE AGING AS OSTEOARTHRITIS THERAPEUTICS BY DRUG REPURPOSING

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Background: Effective treatments for Osteoarthritis (OA) are not available. In aging-related diseases, including OA, failure of cellular homeostasis mechanisms, such as autophagy can cause extracellular matrix destruction and cell death (1). With aging, chondrocyte function is diminished, contributing to a cellular senescence phenotype often observed in OA chondrocytes. In addition, a defect in autophagy is observed in both aging and cartilage degeneration (2,3).

Objectives: The objective of this study was to identify anti-senescence and pro-autophagy molecules by a cell-based high-throughput screening (HTS) in human chondrocytes.

Methods: To induce cellular senescence or reduced autophagy, immortalized human chondrocytes (TC28a2) were seeded (2500 cells/well) in 384 well plates, and treated with IL-6 (20 ng/ml) for 72 or 18 hours, respectively. Then, chondrocytes were incubated with Prestwick Chemical Library (1120 approved drugs with chemical and pharmacological diversity, as well as bioavailability and safety in humans) at 10 μ M for 72 hours. To identify anti-senescence hits, nuclei was stained with Hoechst 33342 (2.5 μ g/ml), while β -galactosidase subcellular structures was stained by using Image Green C12FDG substrate (30 μ M). To evaluate autophagic flux, a reporter cell line was generated by retrovirus transfection of pBABE-mCherry-EGFP-LC3 plasmid in TC28a2 chondrocytes. Plates were imaged by using Operetta® High Content Screening (HCS) system in non-confocal mode using the 20x WD objective. For each well, 4 fields and 4 planes of bright field, Hoechst and fluorescein channels were obtained. Relative intensity of C12FDG in cytoplasm and number of autophagosomes/autolysosomes per area of cytoplasm were determined to quantitate β -galactosidase activity and autophagy flux respectively. Compound validation was performed in TC28a2 chondrocytes and in primary human chondrocytes by evaluating cell senescence, autophagy pathway and cell death by apoptosis.

Results: A primary screening was performed to identify anti-senescence compounds by measurement of senescence-associated β -galactosidase activity. 283 compounds with anti-senescence effects were identified. The anti-senescence hits were analyzed by monitoring autophagic flux. 29 compounds with both anti-senescence and pro-autophagy effects were selected. Then, one compound was selected for further validation. The compound reduced chondrocyte senescence, increased autophagy ($p<0.0001$) and protected against inflammation and cell death by apoptosis in human chondrocytes ($p<0.05$) in response to IL-6. Interestingly, this protective effect was partially mediated by mTOR inhibition, a proposed mechanism to prevent cartilage aging.

Conclusions: Our screening methodology provides a unique opportunity to repurpose drugs and mechanisms to prevent cartilage aging. Autophagy activation and protection against senescence in chondrocytes may provide benefits for delaying cartilage degeneration.

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