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FRI0512 RETRO-INVERSO TAT-BECLIN-1 INDUCES SYNOVIAL FIBROSIS AND DOES NOT PROTECT CARTILAGE FROM DEGENERATION IN A MOUSE MODEL OF OA

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Background: Beclin-1 is a component of the autophagy pathway necessary for formation of autophagosomes, contributing to autophagy-mediated cellular homeostasis. Enhancing autophagy through inhibition of mTOR activity, either via genetic deletion in chondrocytes or intra-articular injection of rapamycin, attenuates progression of surgically-induced models of osteoarthritis (OA). Retro-inverso TAT-Beclin-1 is a cell-permeable peptide which competes for binding to the endogenous Beclin-1 inhibitor GAPR-1, thus promoting autophagy. It is unknown whether activation of Beclin-1 is sufficient to protect joints from osteoarthritis progression.
Objectives: For this study, we sought to determine if retro-inverso TAT-Beclin-1 could attenuate OA progression in a surgically-induced mouse model.
Methods: Eight-week old C57BL/6 mice underwent destabilization of the medial meniscus (DMM) surgery to induce OA, or sham surgery as a control. Mice were injected intra-articularly with retro-inverso TAT-Beclin-1 (2 mg/kg in 5µl) twice weekly for 2 or 9 weeks. Mice were sacrificed at 10-weeks post-surgery. Knee joints were stained with Safranin-O/Fast-green to evaluate cartilage degeneration and Masson's trichrome to determine degree of synovitis using OARSI scoring for mice. Sections were stained for α-SMA (myofibroblast) and CD45 (hematopoietic-origin cell) to evaluate changes in markers of fibrosis and inflammation, respectively.
Results: As opposed to the effects of mTOR deletion in cartilage or rapamycin treatment in joints, injection of retro-inverso TAT-Beclin-1 for 2 into knee joints of mice with DMM-induced OA had no effect on the degree of articular cartilage degeneration in the tibia or femur as compared to PBS-injected controls. However, in both sham and DMM mice, retro-inverso TAT-Beclin-1 for 2 or 9 weeks of treatment induced a pronounced thickening of the synovium with increased cell numbers and collagen deposition compared to PBS-treated mice. The increased number of synovial cells in 9-week treated mice did not show substantial expression of α-SMA+ or CD45+ cells, suggesting the increased number of cells and matrix in the synovium was independent of myofibroblast differentiation or inflammatory influx.
Conclusion: Contradictory to our expected results, retro-inverso TAT-Beclin-1 did not attenuate cartilage degeneration. Rather, it promoted substantial synovial thickening that likely involved cell proliferation and collagen deposition. This severe fibrotic phenotype appears independent of myofibroblast differentiation or inflammation, normally associated with typical fibrotic responses. To evaluate the potential for dose responses with retro-inverso TAT-Beclin-1 in synovial joints, we are currently modifying our dosing strategy in an effort to determine possible disease modifying effects of this novel Beclin-1 activator.
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FRI0513 HISTONE-ACETYLTRANSFERASES CBP AND P300 REGULATE AUTOPHAGY AND PROTEASOMAL DEGRADATION IN SYNOVIAL FIBROBLASTS

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Background: Proteasomal degradation and autophagy are the major catabolic pathways that maintain the homeostasis of cells and are associated with cell survival. The histone acetyltransferases cAMP-response element binding protein binding protein (CBP) and p300 are close homologues and widely accepted as redundant proteins.
Objectives: To analyse individual functions of CBP and p300 in catabolic pathways in rheumatoid arthritis (RA) synovial fibroblasts (SF).
Methods: SF were isolated from knee, shoulder and hand joints of RA patients undergoing joint replacement surgery. The expression of CBP and p300 was silenced by transfection of antisense LNA gapmeRs (12,5 nM). 24h after transfection cells were stimulated with TNF-α (10 ng/ml, 24h). Transcriptomes were determined by RNA-seq (Illumina NovaSeq 6000, n=6). Pathway enrichment analysis of RNA-seq data (fold change >1.5, FDR <0.05) was performed using DAVID bioinformatic resources. Autophagy was assessed by Western blotting using LC3B conversion and p62 as autophagy markers (n=4) in presence and absence of bafilomycin A1 (100 nM, 4h), a lysosomal inhibitor. Cell death was analysed using the CytoTox-Glo cytotoxicity assay.
Results: The top pathway identified after silencing of p300 in SF in presence ($p=1.33x10^{-10}$) and absence of TNF-α ($p=1.76x10^{-10}$) was 'proteasome', with an enrichment of genes contributing to 'proteasome assembly' and 'proteasome regulation'. The expression of several genes encoding proteasome subunits was increased after p300 silencing but unaffected by silencing of CBP. Genes contributing to the biological processes 'autophagy' ($p<0.05$) and 'regulation of autophagy' ($p<0.05$) were enriched after silencing of CBP and p300, whereas 'autophagy assembly' was only affected by CBP silencing. In RNA-seq data, the expression of MAP1LC3B, encoding the autophagy marker LC3B, and the autophagy substrate p62, was increased by p300 silencing but slightly decreased by CBP silencing. In line with the RNA expression, silencing of CBP reduced the conversion of LC3B and the protein expression of p62 in presence and absence of TNF-α. Results were similar in presence of bafilomycin A1, indicating a decrease in autophagosome synthesis. In contrast, the conversion of LC3B and p62 expression were increased after silencing of p300 in unstimulated SF, indicating increased autophagy. This effect was lost for LC3B after treatment with TNF-α, and LC3B conversion was even decreased in presence of bafilomycin A1. This indicates a late stage block of autophagy after silencing of p300 in TNF-α-stimulated SF. In line with this, silencing of p300 in SF (n=6, $p<0.05$) increased cell death only in presence of TNF-α. Viability of SF was not affected by silencing of CBP.
Conclusion: Here we identified p300 as a major regulator of the proteasome in SF and provide first evidence for individual functions of CBP and p300 in regulating autophagy in SF.
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FRI0514 NEW DIAGNOSTIC BIOMARKER OF BONE TISSUE METABOLISM DISORDERS IN RHEUMATOID ARTHRITIS

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Background: Bone mineral density and proteins/peptides determination in blood and urine as markers of bone resorption and formation are currently used to diagnose osteoporosis (OP) and metabolic bone diseases. However, these methods have some disadvantages for bone turnover evaluation. Recent evidence suggests that in RA changes in the secretion of hormones of white adipose tissue can be revealed [1,2,3,4]. One of them is Adiponectin possessing anti-inflammatory, anti-diabetic and anti-atherogenic properties. Changes in Adiponectin levels may reflect influence of immune inflammation on bone turnover.
Objectives: To study the clinical and diagnostic value of serum Adiponectin determination in RA patients complicated by OP.
Methods: We examined 88 women with documented diagnosis of RA and mean disease duration of 6.56±0.88 years. We used EULAR/ARA 2010 criteria to diagnose the patients. Female patients with II degree of disease activity (DAS28), Steinbrocker stage II (erosive), rheumatoid factor- and anti-cyclic-citrullinated peptide antibody-positive were prevalent. We excluded patients who had surgery or developed an infection within the last 8 weeks, pregnant and breast-feeding women, those with severe heart, liver or kidney disease, immune deficiency, leukopenia or chronic infection. A control group of 45 healthy females aged of 25 and 59 years were included in the study. There were no reported findings of