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FR00512

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 depletion 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-penetrable peptide which competes for binding to the endogenous Beclin-1 inhibitor GAPR1, 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.

Disclosure of Interests: None declared


FR00513

HISTONE-ACYETYLTRANSFERASES CBP AND P300 REGULATE AUTOAPHAGY 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). Transcripts were determined by RNA-seq (Illumina NovaSeq 6000, n=5), 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 bafloycin 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 of TNF-α-SMA+ or CD45+ cells, suggesting the increased number of immune inflammation on bone turnover.

Disclosure of Interests: J Poljakova: Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe. In addition, he had/contributed to research funding from Astellas, Astra Zeneca, Roche, Sanofi; Advisory Board: Biogen, Gilead, Genentech, Genzyme, Lilly, MedImmune, Mitsubishi Tanabe Pharma, Pfizer, Regeneron, Sanofi-Aventis, Septentrio, Shire, UCB; E Papicheva: Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe. In addition, he had/contributed to research funding from Astellas, Astra Zeneca, Roche, Sanofi; Advisory Board: Biogen, Gilead, Genentech, Genzyme, Lilly, MedImmune, Mitsubishi Tanabe Pharma, Pfizer, Regeneron, Sanofi-Aventis, Septentrio, Shire, UCB; V Zavodovskya: Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe. In addition, he had/contributed to research funding from Astellas, Astra Zeneca, Roche, Sanofi; Advisory Board: Biogen, Gilead, Genentech, Genzyme, Lilly, MedImmune, Mitsubishi Tanabe Pharma, Pfizer, Regeneron, Sanofi-Aventis, Septentrio, Shire, UCB; E Papicheva: Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, Bayer, Boehringer Ingelheim and Mitsubishi Tanabe. In addition, he had/contributed to research funding from Astellas, Astra Zeneca, Roche, Sanofi; Advisory Board: Biogen, Gilead, Genentech, Genzyme, Lilly, MedImmune, Mitsubishi Tanabe Pharma, Pfizer, Regeneron, Sanofi-Aventis, Septentrio, Shire, UCB; A Khavrdyay: None declared, Marcel Gabathuler: None declared, Christoph Kolling: None declared, Matija Tomsic: None declared, Oliver Distler Grant/research support from: Prof. Distler received research funding from Actelion, AstraZeneca, Boehringer Ingelheim and Mitsubishi Tanabe to investigate potential treatments of scleroderma and its complications, Consultant for: Prof. Distler has/had consultancy relationship within the last 3 years with Actelion, Bayer, Boehringer Ingelheim, ChemomAb, espeRare foundation, Genentech/Roche, GSK, Inventiva, Italfarmaco, iQvia, Lilly, medImmune, Mitsubishi Tanabe Pharma, Pharmacyclics, Novartis, Pfizer, Sanofi, Seropharma and UCB in the area of potential treatments of scleroderma and its complications. In addition, he had/has consultancy relationship within the last 3 years with A Menarini, Amgen, Abbvie, GSK, Mepha, MSD, Pfizer and UCB in the field of arthritis and related disorders, Caroline Osipelt: None declared, Kerstin Klein: None declared.


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 based cell death only influence the 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 6.5±0.88 years. We used EULAR/AARA 2010 criteria to diagnose the patients. Female patients with II degree of disease activity (DAS28), Steinarbrocker 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, leukaemia or chronic infection. A control group of 45 healthy females aged between 25 and 55 years were included in the study. There were no reported findings of...