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OP0156 METHOTREXATE INCREASES EXPRESSION OF THE CELL CYCLE REGULATORS LBH AND P21 AND REDUCES FIBROBLAST-LIKE SYNOVIOCYTE PROLIFERATION AFTER MITOGEN STIMULATION

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Background: Activated fibroblast-like synoviocytes (FLS) are key effector cells in the joint in rheumatoid arthritis (RA). Local FLS proliferation is responsible for synovial hyperplasia, a key feature of the RA synovium correlating with disease activity. PDGF and IL-1b are known FLS mitogens. LBH is a transcription regulator and tumor suppressor, recently identified as a RA risk gene. We have demonstrated that LBH regulates FLS proliferation and that LBH expression is regulated by growth factors and by epigenetic mechanisms[1]. Methotrexate (MTX) is still the first-line treatment of RA but the target cells and mechanism of action of the low dose used in rheumatic diseases is largely unknown. Increased expression of cell cycle checkpoint genes[2] and modified DNA methylation[3] in immune cells have recently been described.
Objectives: The aim of this study was to investigate the effects of MTX on PDGF and IL1b-induced FLS proliferation *in vitro* and in particular on the expression of LBH, cell cycle genes (CDKN1A/p21 and CCND1/cyclinD1) and on genes regulating DNA methylation (DNMTs) in order to further understand the pharmacodynamics of this drug in RA and to identify novel markers for drug response.
Methods: Primary FLS from RA patients and from patients with osteoarthritis (OA) were plated on day 0 in DMEM complete, pre-treated 24 hours with MTX or control medium day 1, and stimulated with 20ng/ml PDGF+2 ng/ml IL-1b with or without 1 uM MTX in DMEM with 1% FBS for 24–48 hours starting day 2. Cells were then harvested for qPCR for gene expression and flowcytometry for cell cycle analysis.
Results: Stimulating RA-FLS cultures (n=3) with PDGF+IL-1b for 24 hours, pushed 24,5±3,5% cells into G2/M phase compared to 3,4±0,8% in unstimulated controls. Interestingly, treating PDGF+IL1b stimulated FLS with MTX, significantly inhibited cell cycle progression (4,6±1,9% in G2/M phase, p=0,02). PDGF+IL-1b stimulation of FLS for 24 hours reduced LBH mRNA expression. However, in the presence of 1uM MTX the LBH mRNA expression was significantly higher in RA-FLS (3,2±0,5 fold, p=0.002, n=5) and in OA-FLS (2,2±0,5 fold, p=0,02, n=5) after PDGF+IL-1b stimulation compared to untreated controls. In addition, MTX treatment strikingly increased the CDKN1A expression 14,3±4,4 fold (p=0,006) of treated vs untreated stimulated FLS. Furthermore, we found that 1 uM MTX restored and increased a lowered DNMT1 mRNA expression to 144±12% after PDGF+IL1b stimulation. There were no significant effects of MTX on CCND1 or DNMT3a expression at investigated time points.
Conclusions: Therapeutic doses of MTX reduce mitogen induced FLS proliferation and significantly revert mitogen-induced reduction of LBH and p21 expression in RA FLS. MTX restores expression of DNMT1 suggesting that MTX might regulate gene expression and proliferation by affecting the epigenome.
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OP0157 APOPTOSIS RESISTANCE OF SYNOVIAL FIBROBLASTS OF PATIENTS WITH RHEUMATOID ARTHRITIS IS REGULATED BY THE LONG NON-CODING RNA FAS-AS1

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Background: Apoptosis resistance is thought to contribute to the accumulation of synoviocytes in the affected joints of patients with rheumatoid arthritis (RA). Of particular interest, the Fas receptor (FasR) - Fas ligand (FasL) apoptotic pathway appears altered in RA[1]. Long non-coding RNAs (lncRNAs) are emerging as key regulators of gene expression. Their role in disease, however, is still poorly understood. The recently described lncRNA FAS-AS1 has been implicated in alternative splicing of FasR. This results in increased amounts of soluble FasR (sFasR) and thereby prevents FasL-induced cell death[2,3]. Whether lncRNA FAS-AS1 is involved in the apoptosis resistance of synovial fibroblasts in RA is unknown.
Objectives: To assess the regulatory role of lncRNA FAS-AS1 in the apoptosis resistance of synovial fibroblasts from patients with RA (RASF).
Methods: Levels of expression of lncRNA FAS-AS1 were measured in RASF and synovial fibroblasts from patients with osteoarthritis (OASF) by qPCR using SYBRGreen detection. Cells were treated with TNFα (10ng/ml, 24h) and/or with FasL (50ng/ml, 18h) to assess the secreted amount of sFasR by ELISA and the induction of apoptosis by Annexin V staining followed by flow cytometry. LncRNA FAS-AS1 was silenced using locked nucleic acid antisense oligonucleotides (GapmeR).
Results: There was no significant difference in basal levels of lncRNA FAS-AS1 expression between RASF and OASF (n=4 each). TNFα stimulation of synovial fibroblasts, regardless of the disease context (RA or OA), resulted in higher than 6-fold induction of lncRNA FAS-AS1 expression (6.45±1.39; $p<0,05$; n=4 for RASF and 6.26±1.47; p=0.05; n=4 for OASF). In addition, TNFα stimulation induced secretion of sFasR in RASF from 107±74 to 390±274pg/ml ($p<0.05$; n=6) and OASF from 69±54 to 249±134pg/ml ($p<0.05$; n=6). FasL induced apoptosis in both RASF and OASF (55–75±13%). However, pretreatment with TNFα reduced the FasL-induced apoptosis in RASF by 25±19% and in OASF by 15±10%. Silencing with GapmeR successfully decreased the expression of lncRNA FAS-AS1 by 40±22% SEM. Most interestingly, silencing of lncRNA FAS-AS1 reverted the TNFα inhibitory effect on FasL-induced apoptosis by 37±11% (n=4).
Conclusions: Our data revealed a novel mechanism, which may underlie apoptosis resistance in RASF. We showed that in a pro-inflammatory cytokine milieu, lncRNA FAS-AS1 up-regulates the release of sFasR and thereby may lower the responsiveness of cells to death signals. Thus, targeting lncRNA FAS-AS1 might prevent apoptosis resistance and synovial hyperplasia in RA.
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OP0158 PREVALENCE OF IMMUNIZATION OF PATIENTS WITH AUTOIMMUNE DISEASE IN MEXICO

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Background: Current guidelines recommend immunization in patients with autoimmune diseases and use of immunosuppressants including biological treatment. Despite the above, the frequency of immunization is unknown in our population.
Objectives: To identify the prevalence of immunization in patients with autoimmune disease in a Rheumatology Service of a third level hospital in Mexico.
Methods: Observational, descriptive, cross-sectional study. Consecutive outpatients with autoimmune diseases who attended the Rheumatology Service of the Hospital Civil of Guadalajara during a period of 2 months (Dec. and Jan.) were included. A questionnaire was carried out to obtain demographic and immunization data. Descriptive statistical analysis was performed.
Results: 1208 patients were surveyed, 484 (40%) had a diagnosis of autoimmune disease; of whom 286 (59%) were on immunosuppressant therapy. 321 patients had a complete immunization during childhood. None of the patients knew what vaccines should by received with their diagnosis. When asked if they had been invited for immunization and from whom, 24 reported that their