counts (α-SMA staining) and of 23 serum inflammatory cytokines/chemokines (Mouse-Cytokine-23-plex, Bio-Rad-Laboratories).
Results: 17-DMAG decreased dermal thickening by 53±3% (p<0.001) (nintedanib by 46±2%,p<0.001), collagen content by 48±5% (p=0.004) (nintedanib by 50±4%,p=0.003), myofibroblast counts by 42±9% (p<0.001) (nintedanib by 44±7%,p<0.001), and levels of IL-1α, IL-6, IL-12(p40), CXCL1, MCP-1, MIP-1α/β, RANTES (in all: p<0.05) compared to vehicle-treated mice injected with bleomycin for 6w. Moreover, 17-DMAG also induced regression of pre-established fibrosis to below the levels of vehicle-treated mice injected with bleomycin for 3w and NaCl for 3w (dermal thickness by 14±3%, collagen content by 20±5%, myofibroblast counts by 13±9%; whereas in nintedanib by 10±3%, 21±4%, 17±7%, respectively; in all: p<0.05), and levels of IL-12(p40), CXCL1, MCP-1, MIP-1β, RANTES (in all: p<0.05). No significant weight loss, decrease in activity or changes in fur texture were observed upon 17-DMAG treatment.
Conclusion: This is the first study on effects of Hsp90 inhibitor 17-DMAG in the treatment of established dermal fibrosis. We demonstrate that 17-DMAG effectively prevents the progression and induces regression of established bleomycin-induced dermal fibrosis, in an extent that was comparable to nintedanib in this study (which was recently FDA approved for slowing the rate of decline in lung function in adults with SSc-ILD). 17-DMAG was well tolerated without obvious clinical signs of toxicity. These data suggest that Hsp90 could be a novel potential target in the treatment of SSc dermal fibrosis.
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OP0136 THE INFLUENCE OF LONG-TERM EXERCISE AND IN VITRO EXERCISE-MIMICKING STIMULATION ON THE PRODUCTION OF MYOKINES AND CYTOKINES IN MYOTUBES OF PATIENTS WITH CHRONIC IDIOPATHIC INFLAMMATORY MYOPATHIES

M. Vokurková[1], L. Vernerová[1], M. Špiritović[2], H. Štorkánová[3], S. Oreska[3], M. Klein[3], J. Ukropec[4], B. Ukropcová[5], M. Tomčík[3], J. Vencovský[3]. *[1]Institute of Rheumatology, Prague, Czech Republic; [2]Institute of Rheumatology and Department of Physiotherapy, Faculty of Physical Education and Sport, Charles University, Prague, Czech Republic; [3]Institute of Rheumatology and Department of Rheumatology, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic; [4]Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia (Slovak Republic); [5]Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences and Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia (Slovak Republic)*

Background: It has been demonstrated several times that endurance exercise has beneficial effects on the condition of patients with idiopathic inflammatory myopathies (IIM). Muscle contraction during exercise is a major stimulus for the release of myokines that are supposed to take part in the beneficial adaption to exercise.
Objectives: The aim of this study was to find out how a six-month physiotherapy and *in vitro* exercise-mimicking treatment affect myokine and cytokine production in myotubes of IIM patients.
Methods: Seven patients with chronic IIM took part in a six-month physiotherapy (stretching and strengthening), which significantly improved their muscle strength and endurance. IIM patients (n=7) before and after the six months exercise and their respective healthy counterparts (HC, n=9) underwent a *musculus vastus lateralis* biopsy. Isolated skeletal muscle cells were grown, differentiated into myotubes, which were treated with a pharmacological cocktail: palmitate, forskolin and ionomycin (PFI) to mimic exercise-stimulated contractions in vitro. Myokine and cytokine concentrations produced by myotubes to the culture medium were analyzed with ELISA and the multiplex immunoassay, respectively. RT-PCR was used for the evaluation of myokine gene expression in the cultured myotubes.
Results: Compared to myotubes of healthy controls, myotubes of IIM patient released more myostatin and activin A into the medium. The myostatin gene was expressed significantly more in muscle cells of patients than in healthy controls' cells (p<0.05). After a six-month rehabilitation program, activin A secretion was four-fold reduced in myotubes of patients with IIM, while myostatin release and gene expression remained unchanged. In myotubes of IIM patients, less follistatin and more follistatin like 3 were detected in the culture medium compared to HC myotubes. Myotubes derived from IIM patients after six months of rehabilitation secreted twice as much follistatin and half the amount of follistatin like 3 into the medium than myotubes derived from IIM patients prior to rehabilitation (p<0.05). There was no difference in secretion of interleukin (IL) 6, IL-17, tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF) between myotubes of IIM patients and myotubes of HC. However, six-month exercise significantly (p<0.05) reduced release of IL-6, TNF and VEGF in myotubes of IIM patients. Contrary to our expectation, stimulation of PFI had no effect on the release of myostatin, activin A, follistatin and follistatin like 3, or the expression of their genes. PFI treatment significantly (p<0.05) increased IL-6 secretion in myotubes from HC and IIM patients prior to six months of rehabilitation. On the other hand, it was observed that myotubes of HC and IIM patients exposed to the PFI cocktail secreted significantly less inflammatory cytokines IL-17, TNF and VEGF into the medium compared to unstimulated myotubes (p<0.05).
Conclusion: In conclusion, long-term exercise influenced the production of myokines and decreased release of inflammatory cytokines in myotubes of IIM patients. *In vitro* exercise-mimicking treatment increased the secretion of IL-6 and decreased the release of inflammatory cytokines as IL-17, TNF-α and VEGF in myotubes of patients with IIM and healthy individuals.
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OP0137 GENOME-WIDE WHOLE-BLOOD TRANSCRIPTOME PROFILING IN A LARGE EUROPEAN COHORT OF SYSTEMIC SCLEROSIS PATIENTS

L. Beretta[1], G. Barturen[2], B. Vigone[1], C. Bellocchi[1,3], N. Hunzelmann[4], E. Delanghe[5], L. Kovács[6], R. Cervera[7], M. Gerosa[3], R. Ortega Castro[8], I. Almeida[9], D. Cornec[10], C. Chizzolini[11], J. O. Pers[10], Z. Makowska[12], A. Buttgereit[12], R. Lesche[12], M. Kerick[13], M. Alarcon-Riquelme[2], J. Martin Ibanez[13]. *[1]Scleroderma Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [2]GENYO, Centre for Genomics and Oncological Research Pfizer, University of Granada, Andalusian Regional Government, PTS GRANADA, Granada, Spain; [3]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy; [4]Klinik und Poliklinik für Dermatologie und Venerologie, Köln, Germany; [5]Division of Rheumatology, University Hospitals Leuven and Skeletal Biology and Engineering Research Center, Leuven, Belgium; [6]Department of Rheumatology and Immunology, University of Szeged, Szeged, Hungary; [7]Department of Autoimmune Diseases, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [8]IMIBIC, Reina Sofia Hospital, University of Cordoba, Cordoba, Spain; [9]Serviço de Imunologia EX-CICAP, Centro Hospitalar e Universitário do Porto,, Porto, Portugal; [10]1U1227, Université de Brest, Inserm, Labex IGO, CHU de Brest, Brest, France; [11]Immunology & Allergy, University Hospital and School of Medicine, Geneva, Geneva, Switzerland; [12]Bayer AG, Berlin, Germany; [13]Institute of Parasitology and Biomedicine López-Neyra, IPBLN-CSIC, Granada, Spain*

Background: The analysis of annotated transcripts from genome-wide expression studies data is of paramount importance to understand the molecular phenomena underlying the occurrence of complex diseases, such as systemic sclerosis (SSc).
Objectives: To perform whole-blood transcriptome and pathway analysis on whole-blood (WB) RNA collected in two cohorts of European SSc patients. Via a discovery and validation strategy we aimed at characterizing the molecular pathways that differentiate SSc from controls and that are reproducible in geographically diverse populations.
Methods: WB samples from 252 controls and 162 SSc patients were collected in RNA stabilizers. Patients were divided into a discovery (n=79; Southern Europe) and validation cohort (n=83; Central-Western Europe). RNA sequencing was performed by an Illumina assay. Functional annotations of Reactome pathways were performed with the FAIME algorithm. In parallel, a immunophenotyping analysis on 28 circulating cell populations was assessed. We then tested: the presence of differentially expressed genes or pathways and the correlation between absolute cell counts and RNA transcripts/FAIME scores in regression models. Results significant in both populations were considered as replicated.
Results: A total of 15224 genes and 1277 related functional pathways were available for analysis. Among these, 99 genes and 225 pathways were significant in both sets. The heatmap in figure shows the relative expression of replicated pathways and the distribution of cases and controls (red and green bars). Among the significant pathways we found a deregulation in: type-I IFN, TLR-cascade and signalling, function of the tumor suppressor p53 protein, platelet degranulation and activation. Correlation analysis showed that the count of several cell

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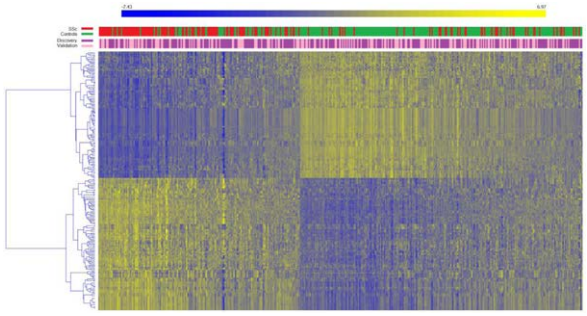
subtypes is jointly associated with RNA transcripts or FAIME scores with strong differences in relation to the geographical origin of samples; neutrophils emerged as the major determinant of gene expression in SSc-whole-blood samples.
Conclusion: We discovered a set of differentially expressed genes and pathways that could be validated in two independent sets of SSc patients highlighting a number of deregulated molecular processes that have relevance for the pathogenesis of autoimmunity and SSc.

Figure:



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OP0138 CLUSTERIN ASSOCIATES WITH DISEASE MECHANISMS AND INFLAMMATION IN MYOSITIS PATIENTS

T. Kropackova[1,2], L. Vernerová[1], H. Štorkánová[1,2], V. Horvathova[1,2], M. Vokurková[1], M. Klein[1,2], S. Oreska[1,2], M. Špiritović[1], B. Heřmánková[3], K. Kubinova[1,2], L. Andres Cerezo[1], O. Kryštůfková[1,2], H. Mann[1,2], J. Ukropec[4], B. Ukropcová[4], J. Zámečník[5], M. Tomčík[1,2], J. Vencovský[1,2], L. Šenolt[1,2].
[1]Institute of Rheumatology, Prague, Czech Republic; [2]First Faculty of Medicine, Charles University, Department of Rheumatology, Prague, Czech Republic; [3]Faculty of Physical Education and Sport, Charles University, Department of Physiotherapy, Prague, Czech Republic; [4]Biomedical Research Center, Slovak Academy of Sciences, Institute of Experimental Endocrinology, Bratislava, Slovakia (Slovak Republic); [5]2nd Medical School and University Hospital Motol, Charles University, Department of Pathology and Molecular Medicine, Prague, Czech Republic

Background: Idiopathic inflammatory myopathies (IIM, myositis) are a heterogeneous group of autoimmune muscle disorders characterized by skeletal muscle weakness and damage, inflammation and extramuscular manifestations. Recent findings suggest that immunological as well as nonimmunological processes, such as endoplasmic reticulum stress, hypoxia, mitochondrial and metabolic dysfunction are involved in the pathogenesis of IIMs [1]. Clusterin (CLU) has been reported to play a protective function in the development of tissue injury, inflammation and autoimmunity, and is involved in the maintenance of immune homeostasis [2].
Objectives: This study aimed to explore a potential involvement of the circulating levels and skeletal muscle expression of CLU in pathogenic mechanisms of IIM.
Methods: A total of 85 IIM patients and 86 healthy controls (HC) were recruited. In addition, 20 IIM patients and 21 HC underwent a muscle biopsy. Circulating concentrations of CLU were measured by ELISA. Serum cytokine profile of patients and HC was assessed by Cytokine 27-plex Assay. Immunohistochemical localisation of CLU was assessed in 10 IIM and 4 control muscle tissue specimens. The expression of CLU and myositis related cytokines in muscle tissue was determined by real-time PCR.
Results: We observed a significant increase of circulating CLU in all IIM patients compared to HC (86.2 (71.6-99.0) vs. 59.6 (52.6-68.4) µg/mL, $p < 0.0001$). Moreover, CLU serum levels were positively correlated with myositis disease activity assessment (MYOACT) ($r = 0.337$, $p = 0.008$), myositis intention-to-treat activity index (MITAX) ($r = 0.357$, $p = 0.004$) and global disease assessment evaluated by physician ($r = 0.309$, $p = 0.015$). In addition to that, a multivariate redundancy analysis revealed a combined effect of serum CLU and cytokine profile (represented by cytokines and chemokines known to be involved in IIM) on disease activity measures. In muscle tissue, CLU mRNA was significantly increased in IIM patients compared to controls ($p = 0.032$) and correlated with IL-1β ($r = 0.489$, $p = 0.034$), IL-6 ($r = 0.581$, $p = 0.009$), TNF ($r = 0.485$, $p = 0.035$) and PGC-1α ($r = 0.709$, $p = 0.001$) mRNA. Immunohistochemistry revealed CLU accumulation in the cytoplasm of regenerating myofibers.
Conclusion: Our results show an up-regulation of clusterin in circulation and skeletal muscle of IIM patients that associates with disease activity and inflammation, and its specific expression in regenerating myofibres. Based on our data and the known cytoprotective function of CLU we suggest an attempt of the organism to limit further muscle damage induced by myositis disease mechanisms.
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OP0139 FUNCTIONAL EVALUATION OF THE SJÖGREN'S SYNDROME AND SYSTEMIC LUPUS ERYTHEMATOSUS DDX6-CXCR5 RISK INTERVAL

M. M. Wiley[1], B. Khatri[1], K. L. Tessneer[1], M. L. Joachims[1], A. M. Stolarczyk[1], A. Rasmussen[1], S. J. Bowman[2], L. Radfar[3], R. Omdal[4], M. Wahren-Herlenius[5], B. M. Warner[6], T. Witte[7], R. Jonsson[8], M. Rischmueller[9], P. M. Gaffney[1], J. A. James[1], L. Ronnblom[10], R. H. Scofield[3], X. Mariette[11], W. F. Ng[12], K. L. Sivils[1], G. Nordmark[10], B. Tsao[13], C. Lessard[1]. *[1]Oklahoma Medical Research Foundation, Oklahoma City, United States of America; [2]University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; [3]University of Oklahoma, Oklahoma City, United States of America; [4]University of Bergen, Bergen, Norway; [5]Karolinska Institute, Stockholm, Sweden; [6]National Institute of Dental and Craniofacial Research, Bethesda, United States of America; [7]Hannover Medical School, Hannover, Germany; [8]Haukeland University Hospital, Bergen, Norway; [9]University of Adelaide, Adelaide, Australia; [10]Uppsala University, Uppsala, Sweden; [11]Université Paris-Saclay, Le Kremlin Bicêtre, France; [12]NHR Biomedical Research Centre, Newcastle upon Tyne, United Kingdom; [13]Medical University of South Carolina, Charleston, United States of America*

Background: Sjögren's Syndrome (SS) and Systemic Lupus Erythematosus (SLE) are distinct chronic, complex autoimmune diseases with shared characteristics such as autoantibodies, heightened interferons, and polyarthritis. SS and SLE genome-wide association studies (GWAS) report strong associations with the *DDX6-CXCR5* risk interval. DDX6 suppresses interferon stimulated gene expression and CXCR5 regulates T cell functions implicated in autoimmunity.
Objectives: To identify functional variants that impact regulation in the *DDX6-CXCR5* interval.
Methods: Fine-mapping was done using ImmunoChip data from 3785 SLE, 1916 SS cases and 6893 population controls of European ancestry that were imputed and tested for SNP-trait association. Bayesian statistics assigned posterior probabilities to SNPs and defined a credible set of risk variants. Bioinformatic analyses further prioritized variants with predicted functionality. Electrophoretic mobility shift assays (EMSAs) and luciferase expression were used to validate predicted SNPs in EBV transformed B (EBV B) cells.
Results: While some differences were observed, the overall SS and SLE association signals were similar. SNP-SS rs9736016 near *CXCR5* and SNP-SLE rs76409436 near *DDX6* were the most significant but did not show evidence of functionality. Bayesian statistics defined credible sets of variants in strong D' in common between both SS and SLE. Bioinformatics analyses (Haploreg, RegulomeDB, ENCODE data, etc) further refined the credible set and identified 5 common SNPs with strong evidence of functionality in immune cell types: rs4938572, rs4936443, rs57494551, rs7117261 and rs4938573. EMSAs showed a significant increase in protein binding to the risk allele of rs57494551 ($p=0.0001$), rs7117261 ($p=0.0001$) and rs4938573 ($p=0.0003$), but not the others, using nuclear lysates from EBV B cells. Luciferase vectors with a minimal promoter or no promoter were used to test for enhancer or promoter activity, respectively. To this end, the rs57494551 risk allele exhibited a significant increase in enhancer activity ($p=0.0001$). In contrast, the rs7117261 risk allele decreased enhancer activity

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