FRI0355 IL-17A UP-REGULATION IN PERIPHERAL BLOOD MONONUCLEAR CELLS CO-CULTURED WITH AUTOLOGOUS SKIN FIBROBLASTS IS ASSOCIATED WITH DOWN-REGULATION OF PRO-FIBROTIC MEDIATORS AND INCREASED FIBROBLAST APOPTOSIS

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Background: IL-17A has been implicated in the pathogenesis of systemic sclerosis (SSc) (1). We previously showed that skewed peripheral blood mononuclear cells (PBMCs) from SSc patients can induce Fas-mediated apoptosis in co-cultured autologous skin fibrobalsts (2).
Objectives: We therefore aimed to investigate IL-17A expression and effects in these co-cultures.
Methods: PBMCs and skin fibroblasts from 5 dcSSc patients with disease duration <3 years were co-cultured up to 10 days in presence of hrIL-2 [20 U/ml] in a 1:10 ratio, as previously described. IL17A, IL17RA, CXCL1, CCL2, CCL3, TGFBR2, SMAD3, CTGF, COL1A1, COL3A1 mRNA expression was assessed by Sybr Green real-time PCR. Chemokine production was further investigated at the protein level by multiple suspension immunoassay. In subset experiments, co-cultures were treated with either IL-17A or IL-17A *plus* anti-IL17 receptor A neutralizing monoclonal antobodies (anti-IL-17RA mAb), then cells were stained with Annexin V, anti-IL17RA, and anti-FAS antibodies and were investigated by flow-citometry.
Results: IL17A mRNA in co-cultured PBMCs was increased by 11.5 fold ($p<0.01$), and IL17RA by 4.3 fold ($p<0.05$) in co-cultured fibroblasts. CXCL-11, CCL2, and CCL3 were also up-regulated at both mRNA (11.9 fold, 773.3 fold, and 29 fold, respectively; $p<0.05$) and protein level (8.9 fold, 11.2 fold, and 252.4 fold, respectively; $p<0.05$). Profibrotic mediators, such as COL1A1, COL3A1, and CTGF mRNA expression in co-cultured fibroblasts was reduced to 0.33 fold, 0.24 fold, and 0.31 fold, respectively ($p<0.05$). This effects were associated with mRNA down-regulation of two key effectors of TGF-β signaling, TGFBR2 and SMAD3 to 0.59 and 0.79 fold, respectively. At flow cytometry analysis, we observed a reduction in co-cultured fibroblasts apoptosis by adding IL-17RA neutralizing mAb to IL-17A treated cells (39% to 16.8%; $p<0.05$), as compared to controls treated with IL-17A and isotype controls. Moreover, IL17RA mAb addition also reduced Fas expression in co-cultured fibroblasts as compared to IL-17A treated cells (47.7% to 10.6%; $p<0.05$).
Conclusions: Our results support the role of IL-17A in the pathogenesis of SSc. Furthermore, here we first show that IL-17A up-regulation in co-cultured PBMCs might play antifibrotic effects in autologous skin fibroblasts and might be implicated in fibroblast apoptosis, interphering with the FAS/FASL pathway.
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Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.6051

FRI0356 ANTISENSE LONG NONCODING RNAS ARE DEREGULATED IN SKIN TISSUE OF SSC PATIENTS

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis of skin and multiple organs of which pathogenesis is poorly understood. Here we studied differentially expressed coding and non-coding genes in relation to SSc pathogenesis with a specific focus on antisense non-coding RNAs.
Objectives: Here we studied differentially expressed coding and non-coding genes in relation to SSc pathogenesis with a specific focus on antisense non-coding RNAs.
Methods: Skin biopsy-derived RNAs from fourteen early SSc patients and six healthy individuals were sequenced with ion-torrent and analysed using DEseq2. Protein-coding and non-coding genes annotated in GENCODEV7 were analysed. Significant long non-coding RNAs were independently replicated in a Northern American dataset.
Results: 4901 genes with a fold change >1.5 and a false discovery rate of less than 5% were detected in patients versus controls. Upregulated coding genes clustered in immunological, cell adhesion and keratin-related processes as previously found by microarray studies. Interestingly, 676 deregulated non-coding genes were detected, 257 of which were classified as antisense genes. 42% of these antisense genes had a concurrent deregulated sense gene. The majority of the sense-antisense genes had a similar effect sizes in an independent North American dataset with three genes (OTUD6B-AS1, CTBP1-AS2 and HMGN3-AS1) exceeding the study-wide Bonferroni-corrected ρ-value ($P_{Bonf}<0.0024$, $P_{combined}=1.6x10^{-9}$, $1.7x10^{-6}$, $2.6xx10^{-6}$, respectively). Intriguingly, the correlation of sense-antisense gene pairs deregulated in SSc is stronger than sense-antisense gene pairs not deregulated in SSc ($p<0.001$).
Conclusions: For the first time we highlight that together with coding genes, (antisense) long noncoding RNAs are deregulated in skin tissue of SSc patients suggesting a novel class of genes involved in pathogenesis of SSc.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.6165

FRI0357 MICROPARTICLES AS A BIOMARKER AND A REDOX-DEPENDENT REGULATOR OF NEUTROPHIL ACTIVATION AND PROTEOLYTIC ACTIVITY IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Background: Persistent oxidative stress and unrelenting vascular inflammation are hallmarks of Systemic Sclerosis (SSc). Platelet-derived microparticles (PD-uP) that express a bioactive redox-dependent moiety, HMGB1 accumulate in the peripheral blood of patients with SSc.
Objectives: To verify whether PD-uP might represent a biomarker of SSc clinical involvement and whether their biological actions is regulated by environmental redox.
Methods: Fifty-four patients with SSc were enrolled so far. Twenty healthy controls (HC) matched for sex and age were studied in parallel. PD-uP were characterized and quantified by flow cytometry. Leukocyte features, including expression and distribution of myeloperoxidase (MPO), were assessed by flow cytometry and confocal microscopy. PD-uP ability to regulate neutrophil activation and proteolytic action was assessed *in vitro* in defined redox conditions and *in vivo* upon intravenous (i.v.) injection in immunocompromised NSG mice, and traced at various times based on recognition of the human platelet antigen CD61. Action on fibroblasts derived from SSc patients and HC are being assessed using biochemical and functional assays.
Results: PD-uP are present in the blood of SSc patients. Their concentration is significantly higher than in the blood of HC ($p<0.0001$). A substantially higher fraction of SSc PD-uP express the prototypic DAMP, HMGB1 (70% SSc vs 5% HC). Among SSc patients, those with pulmonary hypertension had a significantly higher concentration of HMGB1+ PDuP ($p=0.002$). In contrast other disease-associated variables, including the extent of fibrosis and the presence of active SSc pattern at the NVC, were not apparently influent. Neutrophils of SSc patients were activated, as demonstrated by the MPO redistribution from the primary granules to the plasma membrane. Moreover, they had a substantially higher ability to degrade fibrin in vitro, suggesting that enzymes at the plasma membrane are bioactive. Circulating neutrophils appeared to be viable and the fraction of cells undergoing apoptosis was similar in SSc patients and HC. The extent of neutrophil activation was associated with the concentration of HMGB1+ PDuP ($p<0.001$). SSc PDuP but not HC PDuP induced MPO redistribution in vitro. The effect was dependent on HMGB1 and increased by oxidizing moieties. Injection in immunocompromised mice resulted in time-dependent association of SSc PDuP to mouse neutrophils, which contextually redistributed MPO at the plasma membrane.
Conclusions: HMGB1 expression on PD-up of SSc patients could help identify functionally relevant population of microparticles, involved in neutrophil activation/function and possibly valuable as a novel biomarker of vascular remodeling.
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Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.3980

FRI0358 99MTC-RHANNEXIN V-128 AS A NOVEL EARLY DIAGNOSTIC MARKER FOR INTERSTITIAL LUNG DISEASE ASSOCIATED WITH SYSTEMIC SCLEROSIS

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Background: Interstitial lung disease (ILD), the primary cause of death in systemic sclerosis (SSc), often occurs early in the disease course, yet only becomes symptomatic when there is already substantial functional impairment and morphologic changes. Thus, there is an unmet need for early diagnosis. Apoptosis is considered the first pathophysiologic event in SSc-ILD. Monitoring of apoptotic processes with nuclear imaging, a sensitive, specific and noninvasive