collectively suggest that the loss of ATM activation in monocytes may contribute to the disease process of SSc, and is possibly due to DNA damage and oxidative stress.

REFERENCES:

- [1] Guo Z, et al. *Science*. 2010;330:517–521.
- [2] Sambo P, et al. *J Invest Dermatol*. 1999;112:78–84.
- [3] Gabrielli A, et al. *Open Rheumatol J*. 2012;6:87–95.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6340

AB0191 ANTI-SSA AND ANTI-JO1 LEVELS IN INTERSTITIAL LUNG DISEASE RELATED TO IDIOPATHIC INFLAMMATORY MYOPATHIES

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Background: The lung is the most frequently involved extramuscular organ in idiopathic inflammatory myopathies (IIMs); the most common form of lung involvement is interstitial lung disease (ILD). Some autoantibodies are strongly associated with ILD and with specific phenotypes and prognosis of ILD. Among myositis-specific auto-antibodies (MSAs), antibodies against aminoacyl-tRNA-synthetases (AsAb) are the strongest predictive factors for ILD, and anti-Jo-1 is the most common AsAb. Among myositis-associated auto-antibodies (MAAs), anti-SSA/Ro52 is frequently found in sera of patients with IIM and ILD, often associated with anti-Jo-1. The coexistence of anti-SSA/Ro52 and anti Jo-1 seems to be related to a more severe and extensive pulmonary fibrosis with higher score in HRCT compared with the patients with only anti-Jo-1 antibodies. Furthermore, some reports suggest that presence of anti-Jo-1 could be a biomarker for good prognosis¹. The significance of antibodies levels for the prognosis of ILD in IIMs was not widely investigated.

Objectives: To investigate the relationship between antibody levels and clinical manifestations, laboratory data, pulmonary function tests (PFTs), disease activity indices in ILD associated to IIMs.

Methods: Among 130 IIMs admitted to Rheumatology Unit of Bari from January 2010 to January 2018, we retrospectively examined 49 patients (40 F; 22 PM, 25 DM, 1 IBM; mean age at ILD onset 51 years, range: 23–83) because of ILD defined by high resolution computed tomography (HRCT). Clinical manifestations, laboratory data, HRCT pattern, PFTs (FVC, FEV1 and DLCO), therapy, disease activity as Manual Muscle Test (MMT-12), Health Assessment Questionnaire (HAQ), Physician Global Assessment (PGA) at ILD onset, were obtained from medical records. Ferritin levels and autoantibodies were detected in serum samples collected at ILD onset. ANA were tested by IIF on HEp-2 cell substrates, MSAs (JO1, EJO, OJ, PL7, PL12, SRP, HMGCR, Mi2, TIF1γ, MDA5, NXP2, SAE) and MAAs (Ro52, Ku, PM/Scl 75–100, RNP, Scl70) by line-blot method. Anti-SSA and anti-Jo1 were also detected by CLIA method. Correlation analysis were run using parametric and non-parametric test according to data distribution.

Results: 45 of 49 (91,8%) patients were positive for MSAs and/or MAAs. 40 of 45 (88%) were positive at least one of MSAs. The double presence of MSAs and anti-Ro52 was observed in 21 of 40 (52,5%), showing anti-Jo1/SSA in most cases (15/21, 71,4%). Among all correlations studied between anti-Jo1 or anti-SSA levels and PGA or PFTs, we found a significantly correlation between anti-Jo1 and PGA ($p=0,03$, $R=0,46$). We didn't find significantly correlation between autoantibodies and ferritin serum levels.

Conclusions: These findings confirm that ILD was associated with autoantibodies positivity. Further studies in larger cohort need to investigate if autoantibodies levels have a prognostic role in global outcome. Unlikely some controversial works in literature, serum ferritin does not seem a biomarker of severity of lung involvement in IIMs.

REFERENCE:

- [1] Labirua A, et al. Interstitial lung disease and idiopathic inflammatory myopathies: progress and pitfalls. *Curr Opin Rheumatol* 2010.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.7400

AB0192

DISCOVERY OF POTENTIAL SKIN BIOPSY BIOMARKERS FOR SYSTEMIC SCLEROSIS BY HIGH-THROUGHPUT PROTEOMIC APPROACHES

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Background: Systemic Sclerosis (SSc) is an autoimmune connective tissue rheumatic disease characterised by three main hallmarks; vasculopathy, immune system abnormalities and fibrosis. It is considered a multisystemic and heterogeneous disease as many organs of the body may be affected and symptoms vary among patients. Up to date, SSc is untreated and its etiology and pathogenesis remain unclear. Early prognosis and diagnosis of the disease are challenging.

Objectives: The aim of this study was to analyse the proteomic profile of SSc patients in order to gain insights into the mechanisms implicated in disease pathogenesis and also discover new biomarkers that would facilitate early prognosis, more accurate diagnosis and therapeutic targeting of SSc.

Methods: Human biopsies were obtained from ten affected and three non-affected skin areas of SSc patients and have been classified based on histological criteria. Biopsies were cryo-pulverised and proteins were extracted, purified, reduced, alkylated and digested by trypsin. Purified peptides were analysed on a Waters Synapt G2Si HPLC instrument operated in ion mobility mode using a UDMSE approach. Data were processed by the Progenesis Q1p and functional annotation analysis was carried out using multiple bioinformatics resources.

Results: Proteomic analysis led to the identification and quantification of approximately 1500 non-redundant proteins per sample. About 400 of these proteins, including interferons and interleukins, are differentially expressed between affected and non-affected samples. Functional annotation analysis of these proteins showed that they are involved in multiple pathways including, antigen processing and presentation, complement, ubiquitin mediated proteolysis and Notch signalling, which are known to be associated with autoimmune diseases and fibrosis.

Conclusions: Using a Mass Spectrometry-based proteomic approach for the analysis of SSc human skin biopsies, we identified a number of proteins that might be involved in the development and pathogenesis of SSc. Interestingly, some of these proteins are differentially expressed in specific histological groups and thus could be considered as potential biomarkers for specific SSc stages.

Acknowledgements: This work has received support from the EU/EFPIA/Innovative Medicines Initiative Joint Undertaking PRECISESADS grant n° 115565, the Cyprus Institute of Neurology and Genetics and Université catholique de Louvain.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.5283

AB0193

SEMAPHORIN4A INDUCES TH17 CYTOKINE PRODUCTION IN SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterised by inflammation, vascular injury and excessive fibrosis in different organs. Different studies have shown that Th17 cells and Th17 cytokines (IL-17, IL-21 and IL-22) play a key role in the pathogenesis of the disease. Semaphorin 4A (Sema4A) is a transmembrane protein that belongs to a large family of proteins initially described as ligands essential for neuronal development. Further studies have shown that they also play a role in other biological processes including the control of immune responses. Importantly, Sema4A has a critical role in the skewing of CD4⁺ T cells towards a Th17 phenotype. However, it is unknown if Sema4A contributes to the elevated number of Th17 cells observed in SSc patients.

Objectives: The aim of this study was to analyse the potential role of Sema4A as a regulator of Th17-skewing in SScs.

Methods: Plasma levels of Sema4A were measured by ELISA. Expression of Sema4A and its receptors PlexinB2, PlexinD1 and neuropilin-1 (NRP-1) was determined by (q)quantitative PCR, western blot and flow cytometry in monocytes and CD4⁺ T cells of healthy donors (HD) and SSc patients. Monocytes were stimulated with Poly IC (5 µg/ml) or CXCL-4 (5 µg/ml) and Sema4A expression

was analysed by qPCR and ELISA. CD4⁺ T cells were stimulated with anti-CD3/anti-CD28 beads (ratio 1 bead: 5 cells) alone or in combination with recombinant human Sema4A (200 ng/ml), in the presence or absence of neutralising anti-NRP1 or PlexinD1 antibodies, and the expression of PlexinB2, PlexinD1, NRP-1 and the production of Th17 cytokines was analysed by qPCR, ELISA and flow cytometry.

Results: Plasma levels of Sema4A were significantly higher in SSc patients compared to healthy controls (HC) and positively correlated ($r=0.611$) with the skin disease severity. Sema4A and PlexinB2 expression was significantly higher in monocytes and CD4⁺ T cells from SSc patients, respectively. Moreover, Poly IC and CXCL-4 significantly up-regulated the expression and secretion of Sema4A in monocytes from SSc patients, and CD4⁺ T cells stimulation with anti-CD3/anti-CD28 beads increased the expression of PlexinB2 and NRP-1 in both HC and SSc patients. Finally, functional assays showed that Sema4A significantly enhanced the expression of Th17 cytokines induced by CD3/CD28 in CD4⁺ T cells from both HC and SSc patients, and the blocking of the Sema4A signalling using neutralising antibodies anti-PlexinD1 and anti-NRP-1 significantly reduced this expression. Importantly, the Sema4A-induced IL-17 secretion was significantly higher in stimulated CD4⁺ T cells from SSc patient compared to HC.

Conclusions: Sema4A signalling is deregulated in SSc patients and plays an important role in Th17 skewing. Therefore, Sema4A and its receptors could be promising therapeutic targets for the treatment of SSc.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.3692

AB0194

SPARC IS ELEVATED IN THE AFFECTED SKIN OF SYSTEMIC SCLEROSIS PATIENTS AND INDUCES THE EXPRESSION OF FIBROTIC GENES IN DERMAL FIBROBLASTS AND MACROPHAGES

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Background: Systemic sclerosis (SSc) is an autoimmune disease characterised by inflammation, vascular injury and excessive fibrosis in multiple organs. SPARC is a matricellular glycoprotein that can bind to extracellular matrix (ECM) components, as well as cellular receptors and secreted growth factors. In doing so, SPARC regulates biological activities dependent upon cellular interactions with the ECM as well as processes dependent upon cell adhesion, including tissue remodelling, wound healing, angiogenesis and immune responses. Several studies have implicated SPARC in the pathology of SSc but the specific role of SPARC in fibrosis is still unknown.

Objectives: The aim of this study was to analyse the potential role of SPARC as a regulator of fibrosis in SSc.

Methods: Expression of SPARC in the skin of healthy donors (HD) and SSc patients was measured by immunohistochemistry. Peripheral blood-derived monocytes from HD and SSc patients were differentiated into macrophages with M-CSF (25 ng/ml). Dermal fibroblasts and M-CSF macrophages from both HD and SSc patients were stimulated with SPARC (0.1 and 1 µg/ml) for 6 hour and 24 hour. mRNA and protein expression of SPARC and other fibrosis-related genes were measured by qPCR and western blot.

Results: We found increased expression of SPARC in the affected skin of SSc patients compared to HD. We also observed a higher expression of SPARC and ECM components (collagen(Col)-1 and fibronectin-1 (FN1) in dermal fibroblasts derived from SSc patients. SPARC stimulation induced mRNA expression of important fibrosis-related genes such as *TGFB1*, *PDGFB*, *SERPINE1* and *CTGF*, and ECM components including *COL1A1*, *COL3A1*, *COL4A1* and *FN1* in dermal fibroblasts from SSc patients, but not healthy donors. In M-CSF macrophages from SSc patients, SPARC also up-regulated mRNA expression of *TGFB1*, *PDGFB*, *STAB1*, *COL1A1* and *FN1*.

Conclusions: These results suggest that SPARC is an important pro-fibrotic mediator contributing to the pathology driving SSc. Therefore, SPARC could be a promising therapeutic target for reducing fibrosis in SSc.

Disclosure of Interest: S. García Pérez: None declared, B. Malvar Fernández: None declared, M. Bergin Employee of: UCB, T. Johnson Employee of: UCB, W. Marut: None declared, K. A. Reedquist: None declared, T. R. Radstake: None declared

DOI: 10.1136/annrheumdis-2018-eular.3702

AB0195

PROTEOMIC APPROACH IDENTIFIES DIFFERENTIAL PROTEIN EXPRESSION IN CULTURED FIBROBLASTS UNDER STIMULATION WITH TGF-β1

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Background: Fibroblasts (Fb) are key effectors cells in systemic sclerosis (SSc).¹ Fb stimulation with TGF-β1 is usually considered as the positive control in studies assessing the fibrogenesis in SSc.² Yet, the lack of standardisation of TGF-β1 stimulation might be responsible for discrepancies in experiments performed in different conditions. Proteomic approach allows the analysis of differential expression of the whole proteins (proteome) in Fb, and appears an interesting approach to compare different culture conditions.

Objectives: We designed this study to compare the whole protein expression in Fb stimulated by TGF-β1 in different conditions.

Methods: At fifth passage, primary culture of human Fb from healthy subjects (ATCC; PCS-201-012) were stimulated or not with different concentrations of recombinant human active TGF-β1 (0.04, 1, and 5 ng/mL) (R and D Systems; 240-B-002) during 24, 48 and 72 hours. Proteins were extracted and analysed using an eFASP LC-MS/MS approach on an Orbitrap mass spectrometer (Thermo Scientific; Q Exactive +). Proteins quantitation was performed by Maxquant and statistical analysis by Perseus using ANOVA and principal component analysis (PCA).

Results: A total of 3267 proteins were identified, of which 1957 showed differential expression using ANOVA analysis. PCA revealed several clusters of differential proteins expression (figure 1). There were clear clusters of protein expression related to (i) unstimulated and stimulated conditions, (ii) between the three different times of stimulation and (iii) to TGF-β1 concentrations used. Although the expression of proteins in Fb exposed to 0.04 and 1 ng/mL of TGF-β1 during 72 hour were rather close, there was a unique proteins profile related to the condition with 5 ng/mL of TGF-β1 during 72 hour.

Figure 1: PCA representation of differential proteins expression in different conditions. [TGF-β1]=0.04 ng/mL: square; [TGF-β1]=1 ng/mL: circle; [TGF-β1]=5 ng/mL: diamond. The more the points appear distanced, the more different is the protein expression.

Abstract AB0195 – Figure 1. PCA representation of differential proteins expression in different conditions.

Conclusions: This study highlights a variation of proteins expression depending on both stimulation time and TGF-β1 concentrations in Fb culture. The identification of protein differentially expressed will provide insights in the impact of TGF-β1 on Fb physiology under stimulation conditions. These data underline the need of standardisation of culture conditions to allow inter-data comparisons using in sensitive “omic” approaches.

REFERENCES:

- [1] Garret SM, Frost DB, Feghali-Bostwick C. The mighty fibroblast and its utility in scleroderma research. *J Scleroderma Relat Disord* 2017;2(2):69–134.
- [2] Verrecchia F, Mauviel A. Transforming growth factor-beta signaling through the Smad pathway: role in extracellular matrix gene expression and regulation. *J Invest Dermatol* 2002 Feb;118(2):211–5.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6633