AB0188

ACTIVATED PLATELETS ARE INCREASED IN CIRCULATION OF PATIENTS WITH SYSTEMIC SCLEROSIS AND ASSOCIATED WITH CLINICAL CHARACTERISTICS

H. Yasuoka¹, K. Sakata¹, K. Yoshimoto¹, K. Yamaoka¹, M. Kuwana², T. Takeuchi¹.
¹Keio University School of Medicine; ²Nippon Medical School Graduate School of Medicine, Tokyo, Japan

Background: Systemic sclerosis (SSc) is a systemic connective tissue disease characterised by excessive fibrosis, microvascular injury and autoantibody production [1], but mechanisms of disease process are still under investigation. Recent studies focused on the role of circulating blood cells in the pathogenesis, especially lymphocytes [2,3] or monocytes [4,5], however other cellular components are not well-examined. Platelets play a significant role in hemostasis physiologically. However, recent studies have revealed that platelets contain various kinds of humoral factors such as cytokines, chemokines and growth factors and can distribute systemically through circulation and contribute to the disease process through activation and release of these factors [6].

Objectives: To elucidate the role of platelets in the pathogenesis of SSc, activation status of circulating platelets in patients with SSc and association with clinical characteristics were examined.

Methods: Twenty-one patients with SSc who fulfilled 2013 ACR/EULAR classification criteria and 16 healthy controls were involved. Platelets or microparticles (MPs) were defined as vesicles in platelet-rich plasma which is more or less than 1 µm in diameter by forward and side scatter, respectively, and positive staining with anti-CD41 antibody using flow cytometry. Activation status of platelets was examined by the expression of activation markers on platelets such as P-selectin (CD62P) or activated glycoprotein IIb/IIIa (PAC1). Production of microparticles (MPs) is defined as ratio of proportion of MP to that of platelets. Release reaction of platelets was evaluated by release of platelet factor 4 (PF4) in culture supernatant of coculture with skin fibroblasts using enzyme-linked immunosorbent assay (ELISA). Association or correlation between proportion of activated platelets and clinical characteristics or parameters of patients with scleroderma was also examined.

Results: As for the characteristics of 21 patients with SSc, male to female ratio was 3: 18, proportion of diffuse cutaneous SSc was 24%, mean age was 63±13 years, and mean disease duration was 16±13 years. In SSc, both proportion of CD62P+ or PAC1 +activated platelets ($p<0.05$, $p<0.05$, respectively) and production of MP were higher ($p<0.05$) compared to those in healthy controls. Of these, proportion of CD62P+platelets and MP production were correlated each other ($r=0.88$, $p<0.05$). Also, release reaction of platelet was upregulated in platelets from patient with SSc compared to healthy controls ($p<0.05$). When activation status of platelets was compared to clinical parameters, both proportion of CD62P +activated platelets and MP production were higher in diffuse cutaneous SSc ($p<0.05$, $p<0.05$, respectively) and correlated with modified Rodnan skin score ($p<0.05$, $p<0.05$, respectively).

Conclusions: In SSc, proportion of activated platelets were higher and associated with skin sclerosis, suggesting the involvement in the pathogenesis of SSc.

REFERENCES:

- [1] Denton CP, et al. *Nat Rev Rheumatol*. 2013;9(8):451–64.
- [2] Almeida I, et al. 2015;49(3):347–69.
- [3] Yoshizaki A. *Immunol Lett*. 2018.
- [4] Moleno-Moral A, et al. *Ann Rheum Dis*. 2018.
- [5] Masuda A, et al. *Arthritis Res Ther*. 2013;15:R74.
- [6] Semple JW, et al. *Nat Rev Immunol*. 2011;11(4):264–74.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6162

AB0189

3D SKIN ORGANOID MIMICKING SYSTEMIC SCLEROSIS GENERATED BY PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS: 'DISEASE IN A DISH' AND DEVELOPMENT OF ANIMAL MODEL

J.-W. Kim¹, Y. Kim², J. Kim², M.-J. Park³, E. Kwon¹, J. Lee¹, S.-K. Kwok¹, J. H. Ju^{1,2}, S.-H. Park¹. ¹Division of Rheumatology, Department of Internal Medicine, School of Medicine, The Catholic University of Korea, Seoul St. Mary's Hospital; ²Catholic iPSC Research Center, College of Medicine, The Catholic University of Korea; ³Rheumatism Research Center, Catholic Research Institute of Medical Science, The Catholic University of Korea, Seoul, Korea, Republic of Ireland

Background: Systemic sclerosis (SSc) is a rare autoimmune disease characterised by vasculopathy and fibrosis of various organs including skin. Although SSc has high morbidity and mortality, evidences for disease modifying treatment are

still lacking due to difficulties in performing clinical trials. Patient-specific induced pluripotent stem cells (iPSCs), which can differentiate into various cell types, are used in 3D organoid formation.

Objectives: We generated 3D skin organoid model from SSc-derived iPSCs by differentiating them into keratinocytes and fibroblasts. SSc-mimicking 3D skin organoid can be used in studies for disease modelling and drug screening.

Methods: Peripheral blood mononuclear cells (PBMCs) from patients with SSc were reprogrammed to iPSCs. SSc-derived iPSCs differentiated into keratinocytes and fibroblasts *in vitro*. Expression of markers for iPSCs, keratinocytes, and fibroblasts were determined by reverse transcription polymerase chain reaction (RT-PCR) analysis and immunofluorescence assay (IFA). 3D skin organoid using iPSC-derived differentiation cell line was generated by 3D culture system. Histologic analysis was performed on 3D skin organoid. SSc-derived 3D skin organoid was applied to SCID skin defect mice. Histologic analysis was also performed on SCID skin graft model.

Results: SSc-derived iPSCs formed colonies that resemble embryonic stem cells. Alkaline phosphatase staining showed undifferentiated state of iPSCs. Expression of iPSC markers was increased on SSc-iPSCs. Differentiated keratinocytes and fibroblasts from iPSCs highly expressed their markers for keratinocytes and fibroblasts, respectively. Dermis of SSc-derived 3D skin organoid was thicker and denser than that derived from healthy control. Epidermis and dermis of SCID skin graft model were thickened in those derived from SSc compared to those derived from healthy control.

Conclusions: Patient-derived 3D skin organoid and animal model well represented the characteristics of SSc. These models can serve as useful research tools to understand the disease and screen new drugs for SSc.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.4502

AB0190

IMPAIRED ACTIVATION OF ATAXIA-TELANGIECTASIA MUTATED PROTEIN KINASE IN IMMUNE CELLS IS ASSOCIATED WITH CLINICAL FEATURES IN PATIENTS WITH SYSTEMIC SCLEROSIS

K. Sakata¹, H. Yasuoka¹, K. Yoshimoto^{1,2}, K. Yamaoka¹, T. Takeuchi¹.

¹Department of Internal Medicine, Division of Rheumatology, Keio University School of Medicine; ²Clinical and Translational Research Center, Keio University Hospital, Tokyo, Japan

Background: Ataxia-telangiectasia mutated (ATM) is a protein kinase associated with ataxia-telangiectasia (AT), which is an autosomal recessive disorder due to defective functional activity of ATM. Telangiectasia, seen in AT, is also well known as one of the major characteristics of systemic sclerosis (SSc). ATM plays an important role not only in DNA damage repairing system, but also in the process of regulation of oxidative stress.¹ Moreover, it has been reported that oxidative stress may contribute to disease process of SSc^{2,3}. Based on these background, we hypothesised that ATM may play a substantial role in the pathogenesis of SSc. However, the possible association between ATM activity and SSc development is not fully understood.

Objectives: To clarify the role of ATM in the pathogenesis of SSc, we demonstrated the expression and activation level of ATM in circulating immune cells and analysed the association with clinical characteristics of the patients.

Methods: Whole blood samples were collected from twenty-four patients with SSc and 12 healthy controls (HC). Expression levels of total ATM and active phosphorylated ATM (pATM) were examined in each immune cell subset (neutrophil, monocyte, T cell, B cell and NK cell) by mean fluorescence intensity (MFI) using flow cytometer. Each MFI level of ATM and pATM was compared between patients with SSc and HC, and was analysed the correlation with clinical characteristics of SSc patients, retrospectively collected from patients' records.

Results: The expression level of pATM was significantly lower in monocytes, neutrophils, and T cells in SSc as compared with HC ($^{1887} \pm 128$ vs 2386 ± 181 , $p=0.03$; 10265 ± 861 vs 16087 ± 1218 , $p=0.0004$; 1326 ± 73 vs 1675 ± 103 , $p=0.009$; respectively), whereas no significant difference in total ATM level was observed in each cell subset between two groups. Notably, the expression levels of pATM in monocytes of the patients with interstitial lung disease (ILD) was lower than that of the patients without ILD ($^{1663} \pm 136$ vs 1999 ± 96 , $p=0.05$). Furthermore, there was a tendency of correlation between pATM level in monocyte and parameters of pulmonary function test, such as forced vital capacity. No significant differences and/or correlation between pATM expression level in other cell subsets and clinical parameters of the patients, such as SSc subtype, SSc-specific autoantibodies, presence of pulmonary arterial hypertension, gastrointestinal involvement, digital tip ulcer, pitting scar and modified Rodnan skin score were observed.

Conclusions: In SSc, phosphorylated level of ATM in monocytes, neutrophils, and T cells was significantly lower than that of HC. Importantly, we found that ATM activation was impaired in monocyte of the SSc patients with ILD. These results

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

M. Giannini¹, M. Tampoia², L. Abbracciavento², F. Girolamo³, A. Lia³, E. Mascolo⁴, D. D'Abicco⁵, L. Coladonato¹, F. Iannone¹. ¹Rheumatology Unit, University of Bari; ²University Clinical Pathology Laboratory; ³D.S.M.B.N.O.S, Neurology; ⁴Hospital Clinical Pathology Laboratory; ⁵Marinaccio General Surgery, Bari, Bari, Italy

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/Sci 75–100, RNP, Sci70) 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

P. Chairta¹, P. Nicolaou², K. Sokratous³, G. Spyrou³, A. Oulas³, C. Galant⁴, F. Houssiau⁵, B. Lauwerys⁵, K. Christodoulou². ¹Neurogenetics Department, Cyprus School of Molecular Medicine, Nicosia, Cyprus; ²Neurogenetics Department; ³Bioinformatics Department, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, Nicosia, Cyprus; ⁴Department of Pathology, Université catholique de Louvain; ⁵Rheumatology Department, Cliniques Universitaires Saint-Luc, Bruxelles, Belgium

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 HDMS instrument operated in ion mobility mode using a UDMS^E 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

S. García Pérez, T. Carvalheiro, A.J. Affandi, B. Malvar Fernandez, I. Dullemond, M. Cossu, W. Marut, K.A. Reedquist, T.R. Radstake. *Laboratory for Translational Immunology (LTI) and Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, Netherlands*

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