Disclosure of Interests: Kristina Clark: None declared, Alice Cole: None declared, Xu Shiw-en: None declared, Voon Ong: None declared, Christopher D Buckley Employee of: founder of Mesast Therapeutics https://mesastherapeutics.com/, Christopher P Denton: None declared.

POS0482 LONG NON-CODING RNA H19X IS A MEDIATOR OF ENDOTHELIAL CELL ACTIVATION IN SYSTEMIC SCLEROSIS

F. Tirelli1,2, F. Pachera2, R. Latyfis3, M. Huang4, S. Assassi5, E. Camarillo2, F. Zulian2, G. Kania2, O. Distler1. 1University of Zurich, University Hospital Zurich, Department of Rheumatology, Center of Experimental Rheumatology, Zurich, Switzerland; 2University of Padova, Department of Women's and Child's Health, Department of Medicine, University of Padova, Italy, Department of Medicine, Division of Rheumatology and Clinical Immunology, Pittsburgh, United States of America; 3University of Texas Health Science Center at Houston, McGovern Medical School, Department of Internal Medicine, Division of Rheumatology, Houston, United States of America

Background: In one of our previous studies, we demonstrated that long non-coding RNA (IncRNA) H19X plays a crucial role in the development of TGFβ-driven fibrosis in systemic sclerosis (SSc) and other fibrotic diseases.

Objectives: To define the functional relevance of H19X in endothelial cell (EC) activation as a decisive process in SSc vasculopathy.

Methods: Correlation between H19X expression and microvascular gene signature was computed on bulk RNA-Seq data derived from SSC skin biopsy specimens of patients enrolled in the multicentre Prospective Registry of Early Systemic Sclerosis cohort (PPR, n=48 SSc vs. n=33 healthy controls, HCa). Single cell RNA sequencing (scRNA-seq) data were collected from 27 diffuse cutaneous SSc (dcSSc) and 10 HC skin biopsy specimens. Single cells were barcoded and encapsulated in droplets using a 10X Genomics system. After CDNA synthesis, the libraries were prepared and sequenced using Illumina NovaSeq-500 platform. Seurat package v 3.0.x was used to perform data analysis. EC were identified by enrichment of EC markers CLDN5, VWF, and PECAM1. One thousand five hundred eighty-three and 3398 EC were identified from HC and SSc patients, respectively. Cells were analysed for the expression of H19X and EC activation markers. Additionally, differential expression and pathway enrichment analysis between H19X expressing cells and H19X negative cells was carried out. The function of H19X was investigated in human dermal microvascular EC (HDMEC) by silencing, using locked nucleic acid antisense oligonucleotides (LNA GapmeRs). Gene expression was measured by qPCR. Protein levels of endothelial adhesion molecules were evaluated by Western Blot. Endothelial adhesion was evaluated by co-culture of HDMEC and fluorescently labelled peripheral blood mononuclear cells (PBMCs).

Results: H19X expression was found significantly upregulated in SSc skin biopsy specimens of the PPR cohort (p<0.0001). The expression of H19X positively correlated with the microvascular endothelial cell gene signature in all subjects (SSc and HC, R=0.43, p<0.0001), confirming that H19X is expressed in this cell type. To determine if H19X might be an important factor in SSc EC dysfunction, scRNAseq was performed. This analysis revealed a significant upregulation of H19X in SSc EC as compared to HC EC (p=0.0095). H19X was found to be upregulated in several EC subclusters including arterial (SEMA3G, HEY1), capillary (CA4, RGCC), venous (ACKR1, VCAM1) and lymphatic (PROX1, LYVE1). H19X displayed highest expression in injured SSC EC and capillary SSC EC. Co-expression analysis of the scRNA-seq data revealed higher expression of several adhesion molecules in EC expressing H19X, including VCAM1, ICAM and JAM3. KEGG pathway analysis revealed that differentially expressed genes in H19X expressing cells were highly associated with the ‘Cell adhesion molecule’ pathway (p=2.20e-7). H19X silencing lead to a significant downregulation of mRNA levels of genes encoding adhesion molecules VCAM1 (n=7, p<0.05) and E-selectin (n=7, p<0.01) at 48h after transfection, VCAM1, but not E-selectin, was also reduced at protein level as revealed by Western Blot (n=3). The functional relevance of H19X on endothelial adhesion was confirmed by PBMCs with H19X silenced HDMEC where we were able to demonstrate a significant decrease in leucocyte-to-endothelial cell adhesion (n=5, p<0.05).

Conclusion: Our results show that IncRNA H19X could contribute to EC activation in SSc vasculopathy, acting as a regulator of expression of adhesion molecules.

REFERENCES:
2 Disclosure of Interests: Francesca Tirelli: None declared, Elena Pachera: None declared, Robert Latyfis Consultant of: Pfizer, Bristol Myers Squibb, Boehringer Ingelheim, Formation, Sanofi, Boehringer-Mannheim, Merck and Genentech/Roche, Grant/research support from: Corbus, Formation, Moderna, Regeneron, Pfizer and Kiniksa, Menqi Huang: None declared, Shadiy older: None declared, Eva Camarillo: None declared, Francesco Zulian: None declared, Gabriela Kania: None declared, Oliver Dietser Speakers bureau: Bayer, Boehringer Ingelheim, Janssen, Medscape, Consultant of: Abbvie, Acceleron, Alcimed, Amgen, Anach, Anx, AstraZestone, Baecon, Blade, Bayer, Boehringer Ingelheim, Corbus, CSL Behring, 4P Science, Galapagos, Glenmark, Horizon, Inventiva, Kymera, Lupin, Mittenly Biotec, Mitsubishi Tanabe, MSD, Novartis, Pro- methèse, Roivant, Sanofi and Topadur, Grant/research support from: Kymera, Mitsubishi Tanabe, Boehringer Ingelheim.

POS0483 THE EFFECT OF VITAMIN D3 AND ß-TOCOPHEROL ACETATE IN THE PRECLINICAL MODEL OF SYSTEMIC SCLEROSIS

B. Doskaluk1, L. Zaits3, R. Yatsyshyn1. 1Ivano-Frankivsk National Medical University, Pathophysiology, Ivano-Frankivsk, Ukraine; 2Ivano-Frankivsk National Medical University, Internal medicine #1 clinical immunology and allergology, Ivano-Frankivsk, Ukraine

Background: The pathogenesis of systemic sclerosis (SSc) is characterized by complex damage of an organism due to the development of mechanisms of autoimmunity. Dysregulation of immune system, development of fibrosis, and vasculopathy play a significant role in this process.

Objectives: This study aimed to investigate the effect of antioxidant ß-tocopherol acetate and immunomodulator vitamin D 3 in the preclinical model of SSc.

Methods: To perform this study, three groups of laboratory animals were formed: a control group (20 animals), an experimental group #1 (25 animals) and an experimental group #2 (25 animals). Experimental animals were mature laboratory rats of the Wistar line weighing 180-220 g. Model of SSc was performed in the experimental group #1 using sodium hyPOCHONITE (NaClIO) according reported previously.[1] Laboratory animals of the control group were injected with the isotonic solution according to the same scheme. In the experimental group #2, in addition to NaClIO, laboratory rats received a solution of ß-tocopherol acetate 10mg/100g of body weight. IV and a solution of vitamin D3 1000 U/100g of body weight. IV for 3 weeks (second half of the experiment). The level of surfactant protein D (Elabscience SP-D ELISA-Kit), the vascular cell adhesion molecule (Elabscience VCAVM-1 ELISA-Kit), and interleukin 13 (Elabscience IL-13 ELISA-Kit) was determined by enzyme-linked immunosorbent assay. ELISA was performed on an enzyme-linked immunosorbent assay STAT FAX 303 plus. Data distribution was evaluated using the Shapiro-Wilk test. Mann-Whitney U test and unpaired t test were used for comparisons between groups, a p-value <0.05 was considered statistically significant.

Results: The level of IL-13 (pg/ml) in the experimental group was higher than in the control (36.4±56.23 vs 9.4±3.4 (p<0.001). The serum concentration of SP-D (pg/ml) among the experimental group of laboratory animals was significantly higher compared to the control subjects 490.20 [228.75–568.73] vs 70.13±33.21, (p<0.05). VCM1-(pg/ml) was also higher in the experimental group VCAVM-1 91.25±143.75 vs 173.7±143.75, (p<0.05). The administration of vitamin D3 and ß-tocopherol acetate has shown a positive effect for all three investigated parameters. We have found a statistically significant difference between the two experimental groups regarding IL-13, SP-D, and VCAVM-1. The SP-D level in the experimental group #2 was 124.9±18.96, which was significantly lower than in the experimental group #1 (p=0.04). The concentration of IL-13 and VCAVM were also lower in the group of vitamins D3 and ß-tocopherol acetate administration (22.88±11, p<0.001 and 38.73±12.13, p=0.02 respectively).

Conclusion: This study provided evidence that administration of vitamins D3 and ß-tocopherol acetate, given in combination, has a beneficial effect on IL-13, SP-D, and VCAVM-1 in the organisms of the experimental animals.

REFERENCES:

Disclosure of Interests: None declared.

POS0484 LUNG ORGANOIDS: A NOVEL APPROACH TO STUDY THE MOLECULAR PATHOLOGY OF PULMONARY FIBROSIS IN SYSTEMIC SCLEROSIS

1 Aarhus University, Biomedicine, Aarhus, Denmark; 2Aarhus University Hospital, Rheumatology, Aarhus, Denmark; 3University College London, Division of Medicine, London, United Kingdom

Background: Pulmonary fibrosis is one of the major manifestations in Systemic Sclerosis (SSc) associated with high mortality. Mesenchymal transformation of the airway epithelial cells has been implicated as one of the causes for...