OP0082 FIBROSIS AND MICROANGIOPATHY ARE THE MAIN HISTOPATHOLOGICAL HALLMARKS OF SCLERODERMA-RELATED MYOPATHY

C. Corallo¹, M. Cutolo², S. Soldano², N. Volpi¹, D. Franci¹, A. Montella¹, C. Chirico¹, R. Nuti¹, N. Giordano¹. ¹Medicine, Surgery and Neurosciences, University of Siena, Siena; ²Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, Genoa, Italy

Background: Systemic sclerosis (scleroderma, SSc) is an autoimmune connective tissue disease characterized by skin and internal organ fibrosis, coupled with widespread vascular pathology. Skeletal muscle involvement in SSc has often been considered as a minor component of the disease associated with disuse.

Objectives: The goal of this study is to identify specific histopathological hallmarks of skeletal muscle involvement in SSc.

Methods: A total of 50 SSc patients presenting clinical, serological and electromyographic (EMG) features of muscle weakness, were enrolled. Patients underwent vastus lateralis biopsy, assessed for individual pathologic features including fibrosis [type I collagen (Coll-I), transforming growth factor β (TGF- β)], microangiopathy [cluster of differentiation 31 (CD31), pro-angiogenic vascular endothelial growth factor A (VEGF-A), anti-angiogenic VEGF-A165b], immune/inflammatory response [CD4, CD8, CD20, human leucocyte antigens ABC (HLA-ABC)], and membranolytic attack complex (MAC). SSc biopsies were compared to biopsies of ($n=50$) idiopathic inflammatory myopathies (IIMs) and to ($n=50$) noninflammatory myopathies (NIMs). Ultrastructural abnormalities of SSc myopathy were also analyzed by transmission electron microscopy (TEM).

Results: Fibrosis in SSc myopathy (90%) is higher compared to IIM (30%, $p<0.05$) and to NIM (15%, $p<0.05$). Vascular involvement is dominant in SSc muscle (95%), and in IIM (75%) compared to NIM (20%, $p<0.05$). In particular, CD31 shows loss of endomysial vessels in SSc myopathy with respect to IIM ($p<0.05$) and to NIM ($p<0.01$). VEGF-A is downregulated in SSc myopathy compared to IIM ($p<0.05$) and to NIM ($p<0.05$), while VEGF-A165b is upregulated in SSc myopathy. The SSc immune/inflammatory response suggested humoral process with majority (80%) HLA-ABC fibrillar neoexpression and complement deposits on endomysial capillaries MAC, compared to IIM ($p<0.05$), characterized by CD4+/CD8+/B-cell infiltrate, and to NIM ($p<0.05$). TEM analysis showed SSc vascular alterations consisting of thickening and lamination of basement membrane and endothelial cell 'swelling' coupled to endomysial/perimysial fibrosis.

Conclusions: The predominant features of SSc-related myopathy are fibrosis, microangiopathy and humoral immunity. However, it is difficult to identify specific histopathological hallmarks of muscle involvement in SSc, since they could be present also in other (IIM/NIM) myopathies.

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OP0083 MOLECULAR TARGETED IMAGING BIOMARKERS FOR PERSONALIZED MEDICINE STRATEGIES IN SYSTEMIC SCLEROSIS-RELATED INTERSTITIAL LUNG DISEASE

J. Schniering¹, M. Benešová², M. Brunner¹, C. Feghali-Bostwick³, R. Schibli², O. Distler¹, C. Müller², B. Maurer¹. ¹Department of Rheumatology, University Hospital Zurich, Zurich; ²Center for Radiopharmaceutical Sciences, Paul Scherrer Institute, Villigen-PSI, Switzerland; ³Division of Rheumatology & Immunology, Medical University of South Carolina, Charleston, SC, United States

Background: Interstitial lung disease (ILD) is a life-threatening complication in systemic sclerosis (SSc). Substantial research progress has identified distinct genomic and molecular subtypes in SSc-ILD and brought molecular targeted therapies within reach. However, personalized medicine approaches are still lacking since clinically applicable tools for individualized patient stratification are not yet available.

Objectives: To assess the possibility of imaging molecular targets as a biomarker for stage-dependent assessment of ILD in the mouse model of bleomycin-induced lung fibrosis.

Methods: Expression of integrin $\alpha_v\beta_3$ and folate receptor β (FR- β) was analyzed in lung tissues from patients with idiopathic pulmonary fibrosis (IPF), SSc-ILD, and healthy controls as well as from bleomycin treated mice and saline treated controls using immunohistochemistry and qPCR ($n=5-11$). SPECT (Single Photon Emission Computed Tomography) or PET (Positron Emission Tomography) was performed at days 3, 7, and 14 after bleomycin instillation using the integrin $\alpha_v\beta_3$ -specific ¹⁷⁷Lu-c(RGDfK)-ligand and the FR- β -specific ¹⁸F-Azafof. Additionally, ¹⁸F-FDG-PET and high resolution CT (HRCT) scans were performed. The specific lung uptake of the radiotracers over time was assessed by *ex vivo* SPECT or PET/CT scans and biodistribution studies.

Results: Expression of FR- β was significantly increased at the mRNA and protein level of SSc-ILD patients as compared to healthy subjects ($p<0.05$), whereas only its gene expression was upregulated in IPF patients ($p<0.01$). Integrin $\alpha_v\beta_3$ was increased at the protein level of both SSc-ILD and IPF patients ($p<0.05$), while its mRNA expression was not significantly altered.

Similarly, in lungs of bleomycin treated mice, but not of controls, FR- β expression was increased time-dependently at the mRNA and protein level with higher expression at day 3 and day 7, the inflammatory stages of bleomycin-induced lung

fibrosis ($p<0.05$). In contrast, expression of integrin $\alpha_v\beta_3$ was upregulated at day 7 and day 14 at the protein, but not at the mRNA level in bleomycin treated mice, and thus not only in the inflammatory but also in the fibrotic stages ($p<0.05$). ¹⁸F-FDG-PET and HRCT detected time-dependent changes of metabolic activity and ILD morphology in bleomycin treated mice. However, compared with these routinely employed unselective imaging techniques, molecular targeted imaging of integrin $\alpha_v\beta_3$ and FR- β successfully visualized ILD and discriminated lung inflammation and/or fibrosis in a time-dependent manner and in correspondence with the expression changes at the tissue level. The specific lung uptake of ¹⁷⁷Lu-c(RGDfK)-ligand and ¹⁸F-Azafof as compared with the unselective uptake of ¹⁸F-FDG in diseased lungs over time was demonstrated by biodistribution studies and *ex vivo* SPECT or PET/CT scans.

Conclusions: Our data suggest that stage-dependent visualization of ILD with radiotracers that target key markers of lung inflammation and/or fibrosis shows promise for clinical application. As opposed to unselective imaging techniques such as ¹⁸F-FDG-PET and HRCT, the introduction of specific imaging biomarkers for individualized management of SSc-ILD patients could represent the first step towards precision medicine.

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OP0084 RESCUE FROM THE FAILING HEART IN SYSTEMIC SCLEROSIS, A NOVEL INSIGHT: TARGETING TGF- β /FRA2-DEPENDENT AUTOPHAGY

M. Stellato¹, M. Rudnik¹, F. Renoux¹, E. Pachera¹, D. Kayalar¹, K. Sotlar², K. Klingel³, J. Henes⁴, P. Blyszczuk⁵, O. Distler¹, G. Kania¹. ¹Department of Rheumatology, University Hospital, Zurich, Switzerland; ²Institute of Pathology, Ludwig Maximilians University, Munich; ³Department of Molecular Pathology; ⁴Division of Rheumatology, University Hospital, Tübingen, Germany; ⁵Department of Clinical Immunology, Jagiellonian University Medical College, Krakow, Poland

Background: The majority of Systemic Sclerosis (SSc) patients have subclinical primary cardiac involvement, which resembles the inflammatory dilated cardiomyopathy (iDCM) with inflammation and fibrosis. Firstly, cellular progenitors of pathological myofibroblasts remain undescribed.

Secondly, autophagy may favor fibrosis through enhanced differentiation of fibroblasts in myofibroblasts

Objectives: To unravel the role of Fos-related antigen 2 (Fra2)/autophagy crosstalk in TGF- β -driven myocardial fibrosis in SSc

Methods: Genetically modified mice overexpressing Fra2 were used. Endomyocardial biopsies (EMBs) from SSc/iDCM patients and hearts from Fra2 tg and control mice were analysed by immunohistochemistry (IHC) and immunofluorescence (IF). Murine myocardial gp38⁺ (podoplanin⁺) stromal cells were sorted and stimulated *in vitro* with TGF- β . The cellular phenotype was assessed by qPCR, IF, stress fiber staining, SIRCOL and contraction assay on sorted cells. The antisense oligonucleotide GapmeR was used to knock-down Fra2

Results: The myocardium of SSc/iDCM patients ($n=10$) showed the typical phenotype of iDCM: increased fibrotic area with extended collagen deposition and enlarged number of pathological myofibroblasts, expressing Fra2 and, interestingly, the autophagy markers LC3B and Atg5. Similarly, Fra2 tg mice showed higher expression of profibrotic markers: α SMA, vimentin and collagen I compared to control mice ($n=5$), as well as the expression of the autophagy markers LC3B and Beclin in fibrotic regions.

Among cardiac stromal cells (Ter119⁺CD45⁺CD31⁺Sca1⁺CD29⁺) the frequency of gp38⁺ cells was significantly higher in Fra2 myocardium compared to control mice. The majority of gp38⁺ cells co-expressed α SMA, vimentin and collagen I together with autophagy markers (LC3B and Beclin, $n=3$). Following *in vitro* stimulation with TGF- β , gp38⁺ cells entered fibroblast-to-myofibroblast transition characterized by increased mRNA and protein levels of α SMA, collagen I, fibronectin ($n=3-6$), α SMA-fiber and stress-fiber formation ($n=3$), increased cell proliferation ($n=4$; $p=0.04$) and contraction capability ($n=2$; $p<0.05$) and enhanced collagen secretion ($n=3$; $p=0.04$). Importantly, Fra2 tg gp38⁺ cells showed the presence of α SMA and stress fibers even without TGF- β stimulation as well as an increased contraction capability compared to control cells.

TGF- β stimulation of control gp38⁺ cells induced the expression of LC3B, Beclin and Atg5 at mRNA and protein level ($n=3-5$). In contrast, TGF- β inhibition caused the downregulation of these markers ($n=3$).

In addition, Fra2 silencing resulted in a decreased differentiation capability of

gp38⁺ cells: mRNA and protein levels of the profibrotic genes α SMA and collagen I were significantly downregulated (n=5; p=0.007). Moreover, Fra2 downregulation impaired the secretion of collagens (n=4; p<0.05) and the formation of α SMA fibers (n=3) in addition to a significant downregulation of mRNA and protein expression of LC3B, Beclin and Atg5 (n=3)

Conclusions: The TGF- β /Fra2 axis fosters an enhanced autophagy flow, leading in turn to the stromal-to-myofibroblast transition. Therefore, targeting this process might be a therapeutic strategy to abrogate fatal cardiac remodeling in SSc

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OP0085 LONGITUDINAL ANALYSIS OF THE GASTROINTESTINAL MICROBIOTA IN SYSTEMIC SCLEROSIS

E.R. Volkmann¹, A.-M. Hoffmann-Vold², Y.-L. Chang¹, J. Jacobs¹, K. Tillisch¹, E. Mayer¹, P.J. Clements¹, J. Hov², M. Kummen², Ø. Midtvedt², V. Lagishetty¹, Ø. Molberg², J. Braun¹. ¹University of California, Los Angeles, Los Angeles, United States; ²Oslo University Hospital, Oslo, Norway

Background: Gastrointestinal tract (GIT) dysfunction affects the majority of patients with systemic sclerosis (SSc). While the etiology of SSc-related lower GIT dysfunction remains elusive, we have recently demonstrated that the SSc disease state is associated with a distinct colonic microbial consortium and that specific genera are associated with SSc-GIT symptoms in a cross-sectional analysis using colonic lavage specimens.¹

Objectives: To evaluate changes in GIT microbial composition over time in relation to GIT symptoms in SSc patients.

Methods: Adults SSc patients not on chronic antibiotic therapy were eligible to participate. Stool specimens were collected at 0, 3, 6, 9, and 12 months. Healthy controls were matched to SSc patients in a 1:1 ratio at baseline. SSc patients completed the GIT 2.0 questionnaire to assess GIT symptom severity at each collection time. The microbiota from these samples were determined by Illumina HiSeq 2500 16S sequencing. Linear Discriminant Analysis Effect Size was used to identify the genera that showed differential expression in SSc versus controls. Mixed models evaluated changes in GIT symptoms and microbial composition over time.

Results: Among 17 patients with SSc (88% Female; Median age 52.1 years), the mean (SD) baseline total GIT 2.0 score was 0.7 (0.6). The majority of participants (71%) provided at least 4 stool samples over the course of the 12-month study. Principal coordinate analysis illustrated significant microbial community differences in SSc versus healthy controls (p=0.001). Consistent with the results of our prior study using colonic lavage specimens,¹ SSc patients had decreased commensal genera, such as *Faecalibacterium* and *Bacteroides* and increased pathobiont genera, such as *Fusobacterium* and *Erwinia* compared with healthy controls (Figure 1). GIT 2.0 scores did not change significantly over the course of the 12-month study for each subject. However, patients with longer disease durations had increased GIT symptoms over time for the total score (P=0.0038), and the following domains: fecal soilage (P=0.0217), social functioning (P=0.0116), emotional well-being (P=0.0474), and constipation (P=0.0039). There was no difference in the course of GIT symptoms over time between patients with limited versus diffuse cutaneous disease. Both the absolute

and relative abundances of specific genera did not change over time within individual subjects. After controlling for age, gender, ethnicity, disease duration and SSc subtype (i.e. limited vs. diffuse), low abundance of *Bacteroides* was associated with increased GIT symptoms over time.

Figure 1. Genus level taxa associated with UCLA-SSc patients versus healthy subjects. Negative and positive effect sizes denote genera decreased (blue) or increased (red) in SSc patients, respectively.

Conclusions: This study is the first to characterize lower GIT microbiota in SSc patients at multiple time points. The findings provide further evidence that lower GIT dysbiosis is a feature of the SSc disease state and that specific genera may contribute to GIT symptoms and serve as potential targets for therapeutic intervention.

References:

[1] Volkmann et al. Arthritis Rheumatol 2016;68:1483.

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OP0086 LONG NONCODING RNA H19X AS A NEW THERAPEUTIC TARGET FOR FIBROSIS

E. Pachera¹, A. Wunderlin¹, S. Assassi², G. Salazar², M. Frank-Bertoncelj¹, R. Dobrota¹, M. Brock³, C. Feghali-Bostwick⁴, G. Gerhard Rogler⁵, G. Dijkstra⁶, T. van Haften⁵, J. Distler⁷, G. Kania¹, O. Distler¹. ¹Center of Experimental Rheumatology, University Hospital Zurich, Zurich, Switzerland; ²Department of Internal Medicine, University of Texas, Houston, United States; ³Department of Pulmonology, University Hospital Zurich, Zurich, Switzerland; ⁴Division of Rheumatology, Medical University of South Carolina, Charleston, United States; ⁵Department of Gastroenterology and Hepatology, University Hospital Zurich, Zurich, Switzerland; ⁶Department of Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, Netherlands; ⁷Department of Internal Medicine 3, University of Erlangen, Erlangen, Germany

Background: Long noncoding RNAs (lncRNAs) are an emerging class of transcripts involved in the regulation of gene expression. We have recently identified a novel lncRNA, H19X, to be upregulated in systemic sclerosis (SSc). We also demonstrated that H19X is a key mediator of TGF β -driven myofibroblast development and extracellular matrix synthesis.

Objectives: To assess whether (1) H19X upregulation in SSc fibroblast is anti-apoptotic and pro-proliferative thereby favoring fibrosis (2) H19X is a regulator of fibrotic diseases in general.

Methods: To study the function of H19X in apoptosis and proliferation of dermal fibroblasts we silenced H19X using locked nucleic acid oligonucleotides (LNA GapmeRs) followed by microarray analysis, qPCR, BrdU cell proliferation assay, Caspase 3/7 apoptosis assay and scratch assay. Cells were treated with 10 ng/ml TGF β . Lung tissues were obtained from patients with SSc and idiopathic pulmonary fibrosis (IPF) undergoing transplantation, and from healthy controls (HC). Resected gut tissue from fibrotic and non-fibrotic areas was obtained from Crohn's disease patients. Non-cancer-affected gut tissue from resections because of adenocarcinoma was used as control. Expression of H19X was analyzed by quantitative (q)PCR.

Results: H19X knockdown followed by microarray analysis (n=5) showed that FAS signaling pathway, cyclins and cell cycle regulation, regulation of cell cycle progression by Plk3, and free radical induced apoptosis were among the