In addition, analysis for transcription factor motif enrichment in the differentially accessible peaks of the ATAC-Seq analysis revealed that motifs for TEAD2, RUNX family, and NFkB2 were less accessible, while motifs for JUN::FOS, NF2L2 and EHF were more accessible due to the chromatin changes. These results indicate that H19X could promote or repress the activity of these candidate transcription factors by changing chromatin conformation at the sites of their consensus sequence.

Conclusion: ATAC-Seq in combination with microarray analysis identified candidate mechanism for the TGF β regulated profibrotic effects of H19 X including direct effects on chromatin organization and on transcription factors associated with fibrotic pathways.

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OP0185

INFLAMMATION-DEPENDENT DECREASED EXPRESSION OF CD52 ON CIRCULATING CD14+ MONOCYTES FACILITATES ADHESION IN SYSTEMIC SCLEROSIS

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Background: During the course of systemic sclerosis (SSc), infiltration of inflammatory cells, including monocytes, into the organs is a major process leading to fatal fibrosis, remodelling and organ dysfunction.

CD52 protein is highly expressed on CD4⁺ T-cells and plays an important role in the modulation of T-cell receptor signalling. Nevertheless, the function of this protein on monocytes is not completely understood.

Objectives: We aimed to functionally investigate the role of circulating CD14⁺ monocytes in the course of SSc with a special focus on monocyte adhesion and the influence of CD52 expression.

Methods: Biopsies from the heart, lungs and skin of SSc patients (n=11, 7, 7 respectively) and healthy controls (HC) (n=10, 7, 9 respectively) were analysed by immunohistochemistry for the presence of CD14⁺ cells. PolyA RNA sequencing of CD14⁺ monocytes isolated from peripheral blood of lcSSc (n=5, age=54.4 ± 6.7), dcSSc patients (n=5, age=51.8 ± 7.2) and age- and sex-matched HC (n=5, age=50.8 ± 9.7) was performed using Illumina HiSeq 4000 platform. Differentially expressed genes were computed using DESeq2 algorithm. Gene ontology and pathway analysis were performed using Metacore software and ShinyApp. Expression of adhesion molecules was confirmed on the protein level using flow cytometry (HC n=8, SSc n=22). Adhesion of CD14⁺ monocytes to immobilized ICAM1 and TNF α -stimulated endothelial cells was checked using the 96-well plate adhesion assay (HC n=8, SSc n=22). CD52 regulation in CD14⁺ monocytes from HC (n=4) was analysed on mRNA level upon stimulation with different concentrations of LPS, IFN γ , IL-4 and IL-13. THP-1 cell lines with overexpression and silencing of CD52 were generated using lentiviral vectors, and further, their adhesion was investigated (n=4).

Results: Immunohistochemistry confirmed higher infiltration of CD14⁺ cells in the heart (p<0.01), lung (p<0.05), and skin (p<0.001) of SSc patients. We detected 1440 differentially expressed genes between dcSSc vs HC and 225 between lcSSc and HC CD14⁺ monocytes respectively (p≤0.01; log₂ ratio≥0.5). Pathway analysis revealed significant alterations in processes important for tissue infiltration, including adhesion. Flow cytometry analysis showed upregulation of adhesion molecules: integrin α_M (CD11b) (p<0.01) and integrin β_2 (CD18) (p<0.01). On the other hand, expression of CD52 was downregulated in SSc patients (p<0.05). SSc CD14⁺ monocytes exhibited increased adhesion both to ICAM1-coated plates (p<0.01) and to TNF α -stimulated endothelial cells (p<0.05). CD52 mRNA

was significantly increased in a dose-dependent manner after anti-inflammatory stimulation (IL-4, IL-13) and significantly decreased after inflammatory stimulation (LPS, IFN γ) (p<0.05). Overexpression of CD52 in THP-1 monocytes decreased adhesion to TNF α -stimulated endothelial cells (p<0.01). Accordingly, silencing of CD52 increased adhesion of THP-1 monocytes (p<0.01).

Conclusion: This is the first report pointing to an increased adhesion of peripheral blood CD14⁺ monocytes to ICAM1 and endothelial cells in SSc. Our results suggest the primary activation of monocytes in peripheral blood, which translates into higher organ infiltration in SSc patients. Finally yet importantly, we characterised a novel function of CD52 molecule on monocytes and its possible contribution during the course of the disease.

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OP0186

LIN-GP38+ STROMAL CELLS ARE KEY EFFECTOR CELLS IN MYOCARDIAL FIBROSIS AND DEFECTS OF THE CONDUCTION SYSTEM

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Background: Cardiac dysfunction is a significant cause of mortality in SSc due to inflammation, vasculopathy and fibrosis. In fibrotic conditions, the excessive number of fibroblasts, myofibroblasts and extracellular matrix in the myocardium may directly or indirectly affect the cardiac conduction system by different mechanisms. Myofibroblasts are the main players in cardiac fibrogenesis, but their origin in SSc remains unknown.

Objectives: To unravel the role of Fos-related antigen 2 (Fra2) in stromal cell activation, cardiac fibrosis and alteration of the conduction system.

Methods: Fra2 transgenic (tg) mice and Rag2^{-/-} Fra2 tg mice were studied. Hearts from Fra2 tg and WT mice were analysed by immunohistochemistry (IHC) and immunofluorescence (IF). Cardiac function was assessed by echocardiography, electrocardiogram (ECG) and telemetry. Myocardial stromal (Ter119 CD45 CD31⁻, hereafter Lin⁻) gp38⁺ stromal cells were sorted and cultured *in vitro*. The cellular phenotype was evaluated by qPCR, α -smooth muscle actin (α SMA) and stress fibre staining, secreted collagens and contraction assay.

Results: Echocardiography of 20-week-old Fra2 tg mice displayed increased ejection fraction (p=0.02) and fractional shortening (p=0.02) with decreased left ventricle end-diameter and end-volume in systole (p=0.006, p=0.008) and in diastole (p=0.007, p=0.008). ECG in conscious mice revealed lower heart rate (HR) (WT 741 ± 33 BPM, tg 644 ± 37 BPM, p<0.0001) and prolonged QRS, ST and QTc parameters (p=0.001, p=0.01, p=0.01), which may predispose to arrhythmias. Telemetry recording showed the presence of atrio-ventricular (AV) blocks that correlated with disease severity (R=0.9, p=0.02). The myocardium of Fra2 tg mice presented mild inflammation and fibrosis with higher expression of collagen (p=0.03, n=7) and periostin (p=0.001, n=7), increased number of α SMA⁺ myofibroblasts and expansion of stromal Lin⁻gp38⁺ cells compared to WT mice (1.4% ± 1.4, n=15 and Fra-2 tg: 6% ± 4.5, n=14, p=0.001). Fibrosis disrupts the conductivity of cardiac tissue. Therefore, we investigated whether these stromal cells might be responsible for fibrosis. The majority of Lin⁻gp38⁺ cells co-expressed α SMA, collagen, ADAM12 and periostin, indicating that myocardial Lin⁻gp38⁺ cells might acquire a myofibroblast-like phenotype. Moreover, gp38 expression correlated with collagen deposition (R=0.9, p<0.0001). The comparison between Lin⁻gp38⁺ cells isolated from WT and Fra2 tg showed that both α SMA total protein and α SMA fibres were increased in Fra2 cells. Importantly, α SMA fibres co-localized with stress fibres, resulting in a faster and stronger contraction capacity of Fra2 cells (p<0.0001, n=3). Proliferation of Fra2 cells was increased compared to WT (p=0.007, n=5), while apoptosis was unchanged (p=0.335, n=5). Interestingly,

Rag2^{-/-}Fra2 tg mice showed no fibrosis or Lin⁺gp38⁺ cell expansion. ECG parameters of Rag2^{-/-}Fra2 tg mice were not changed compared to control mice, indicating that inflammation is necessary to acquire Fra2-driven fibrotic phenotype and defects in the conduction system.

Conclusion: Fra2 overexpression and inflammation foster stromal cell-to-myofibroblast differentiation, leading to cardiac fibrosis and defects of the conduction system. Targeting this process might be a therapeutic strategy for SSc patients with disorders of cardiac involvement.

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OP0187

RITUXIMAB AND CYCLOPHOSPHAMIDE COMPARISON FOR EFFICACY AND SAFETY IN THE PATIENTS WITH SYSTEMIC SCLEROSIS ASSOCIATED WITH INTERSTITIAL LUNG DISEASE

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Background: Cyclophosphamide (CyP) is considered as a drug of choice for the treatment of interstitial lung disease (ILD) in the patients with systemic sclerosis (SSc). However, according to the literature, the use of CyP leads to rather limited and transient improvement of the pulmonary fibrosis. In this context the search for novel, more efficacious agents has been continued, such as attracting much attention rituximab (RTM).

Objectives: To compare the impact of CyP and RTM on SSc clinical manifestation and activity, and the safety of these agents in the open-label prospective non-randomized study.

Methods: 107 patients with the confirmed SSc diagnosis and ILD evidence based on HRCT findings were enrolled into the study. All patients received low-dose and moderate-dose prednisolone regimens. 36 patients (**Group A**) received **parenteral CyP** for 12±6 months at total dose 10.6±5 g (the average age 47±12 years, females 92%, SSc duration 5.0±4.8 years, diffused/localized forms 1.6/1). 71 patients (**Group B**) received **RTM** at total dose 1.43±0.66 g over the follow-up period 13.2±2 months (the average age 46±13 years, females 83%, SSc duration 5.6±4.4 years, diffused/localized forms 1.4/1); to 32 (45%) of them RTM was added to immunosuppressants due to inadequate efficacy of the latter. The time courses FVC(%), modified skin count (mRss, points), activity index (EScSG, points), left ventricle ejection fraction (LVEF,%), mean pulmonary arterial pressure (EchoCG), and cardiac rhythm and conductivity disorders (ECG) were evaluated.

Results: In Groups A and B the therapy was associated with significant decrease in mRss (p=0.009 and 0.001, respectively) and EScSG (p=0.000165 and 0.001, respectively). Increase in LVEF (61.8±7.3 и 63.6±7.3. p=0.02) was observed only in RTM-treated patients.

Evaluation of FVC time course in Groups A and B revealed significant FVC increase (p=0.034 and 0.000045, respectively), with median increment about 5%. In Group A FVC 10% FVC increase was found in the third of the patients thus exceeding respective parameter in Group B (p=0.2). The patient percentage with FVC decrease by ≥10% was similar in both groups.

During the follow-up period no change of the other studied parameters was observed.

The therapy was better tolerated in RTM-treated group: during RTM therapy adverse reactions emerged in significantly lower proportion of the patients (11/14%) compared with CyP-treated group (19/53%), p=0.0000.

Parameters	Group A	Group B
EScSG 1	2.8±2*	2.8±1.8*
M ± SD		
EScSG 2	1.4±1.17*	1.3±1.1*
M ± SD		
mRss 1	11.2±9.8 *	11.3±9.6 *
M ± SD		
mRss 2	7.9±6.8. *	8.0±6.6*
M ± SD		
FVC 1	80.5±20.1*	77.3±20 *
M ± SD		
FVC 2	85.9±20.5*	82.6±21.*
M ± SD		
Δ FVC%	5.4	5.3
	[-0.6; 12.3]	[0.1; 10.2]
FVC increment by ≥10%, n/%	11/31	14/19.7
FVC decrement by ≥10% n/%	2/5.6	4/5.6

Notes: in Parameters column 1 = before treatment, 2 = after treatment; M ± SD = mean value and standard deviation; * = significant difference between the values measured before and after the treatment

Conclusion: Both agents effectively alleviated skin induration and EScSG, and significantly improved FVC. However, CyP use for a year slightly more frequently resulted in clinically significant FVC increase, probably due to low RTM cumulative dose. RTM was better tolerated compared to CyP. The study findings substantiate potential use of anti-B-cell therapy both as a first-line agent for ILD treatment in the patients with SSc, and in the event of CyP inefficacy of poor tolerability, especially in the patients with cardiopathy.

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OP0188

PATHOGENICITY OF FUNCTIONAL AUTOANTIBODIES AGAINST AT1R IN A MOUSE MODEL OF SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is an autoimmune connective tissue disease featured by autoimmunity, fibrosis and vasculopathy. Although many autoantibodies have been detected in the sera of patients with SSc, it is not clear whether they play a role in the pathogenesis of disease. It has been reported that autoantibodies against the angiotensin-II receptor type 1 (AT1R) are present in the sera of SSc patients and are associated with several clinical symptoms of the disease, suggesting that these autoantibodies may act as pathogenic drivers. Recently, our group has developed a novel mouse model for SSc by immunizing mice with human AT1R (hAT1R). From this model we were able to generate functional monoclonal antibodies agonizing AT1R.

Objectives: In the current study, we aim to clarify, whether B cells and antibodies directed against AT1R are involved in the pathogenesis of experimental SSc *in vivo*.

Methods: To investigate the role of B cells in the hAT1R-induced mouse model of SSc, we immunized B-cell deficient mice with hAT1R. Nine weeks after the first immunization, mice were sacrificed and sera and tissues were collected for further evaluation. To investigate the pathogenicity of anti-AT1R antibodies in the disease, monoclonal autoantibodies against hAT1R were applied to the ear of C57Bl/6 mice by single or repetitive injection. Mice were sacrificed 24 hours or 14 days after the first injection for single and repeated application, respectively, and ear and lung tissues were collected for further evaluation.

Results: Compared to the wild type C57Bl/6 mice, hAT1R-immunized B-cell deficient mice were resistant against experimental SSc with regard to autoantibody production, inflammation in the lung and skin, and skin fibrosis. Furthermore, both single and repetitive injection of monoclonal antibodies against hAT1R induced inflammation in ears of mice. Despite this local effect, repetitive injection of anti-AT1R monoclonal antibodies provoked also inflammation in the lung of mice.

Conclusion: Our data demonstrate that i) B cells are indispensable for the pathogenesis of the hAT1R-induced mouse model for SSc and ii) monoclonal antibodies against hAT1R can induce inflammation in mice. Therefore, our results support a role of autoantibodies against AT1R in the pathogenesis of SSc.

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