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 $\pm$ 6.7), dcSSc patients (n=5, age=51.8 $\pm$ 7.2) and age- and sex-matched HC (n=5, age=50.8 $\pm$ 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 $\leq$ 0.01; log₂ ratio $\geq$ 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 $\pm$ 33 BPM, tg 644 $\pm$ 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% $\pm$ 1.4, n=15 and Fra-2 tg: 6% $\pm$ 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,