Results: We detected 1440 differentially expressed genes between dcSSc vs HC and 225 between lcSSc and HC respectively ($p \leq 0.01$; $\log_2$ ratio ≥ 0.5 , figure 1). Among those, in dcSSc 1076 were upregulated (e.g. MMP9, IL1R2, FLT3, MIF, TLR9) and 364 were downregulated (e.g. TGFBR1, CD44, CD244, HLA-DRA, HLA-G). In lcSSc 160 transcripts were upregulated (e.g. CCL2, WNT5B, MMP17) and 65 were downregulated (e.g. KLF11, IRAK2). We identified 123 commonly deregulated genes between SSc subgroups (e.g. CCL3, CD14, IL27, MMP17). Principal component analysis showed close clustering within SSc subgroups and clear separation from healthy controls. Pathway analysis revealed alterations in several biological processes important in fibrogenesis including antigen presentation, MIF-induced immune responses, TGF- β , NOTCH and WNT signalling pathways. qPCR analysis further confirmed differences in gene expression on mRNA level (n HC=8, n SSc=25, $p \leq 0.05$).

Abstract FRI0420 – Figure 1. Volcano plots representing differentially expressed genes in lcSSc vs HC (A) and dcSSc vs HC (B). Red dots stand for significantly deregulated transcripts ($p \leq 0.01$; $\log_2$ ratio ≥ 0.5).

Conclusions: To our knowledge, this is the first global transcriptome analysis of peripheral blood CD14⁺ monocytes in SSc. Our results suggest an initial activation of monocytes in peripheral blood, which might be further translated into novel cellular biomarker of the disease and potentially used for distinguishing between responders and non-responders to a novel treatment in future clinical trials.

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FRI0421 TOLL LIKE RECEPTOR-7/8 ACTIVATION EXACERBATES MURINE EXPERIMENTAL AUTOIMMUNE MYOSITIS

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Background: Type I interferon (IFN)-regulated proteins are upregulated in muscle and skin tissues of patients with idiopathic inflammatory myopathies (IIM).¹ Type I IFN induction might rely upon the activation of toll-like receptors (TLRs).² Specifically, TLR-7/8 is indeed upregulated in infiltrating leukocytes and muscle tissue of IIM patients.³

Objectives: To investigate whether the activation of TLR-7/8 and type I IFN influences the natural history of the IIM.

Methods: Experimental autoimmune myositis (EAM) was induced by injection of the amino-terminal portion of the murine Histidyl t-RNA synthetase (HisRS). Disease activity was compared in the presence or absence of the TLR-7/8 agonist R848 in wild-type mice and in mice that failed to express the IFN α receptor (IFN α R null).

Results: EAM induced by a single intramuscular immunisation with HisRS spontaneously abated after 7–8 weeks. In contrast, the levels of anti-HisRS autoantibodies, endomysial/perimysial leukocyte infiltration and myofiber regeneration persisted until the end of the follow-up period (22 weeks after immunisation) in mice immunised with HisRS in the presence of R848. Myofiber MHC class I molecules were detectable in HisRS + R848 immunised mice only. Muscle MHC expression occurred in parallel with leukocyte infiltration. Type I IFN was necessary for the prolonged autoantibody response to occur and for the spreading of the autoimmune response, as demonstrated using IFN α R null mice.

Abstract FRI0421 – Figure 1. Role of TLR7/8 activation in HisRS-induced myositis

Conclusions: TLR7/8 activation is needed to induce and maintain a systemic autoimmune response against the skeletal muscle. This EAM model reproduces many characteristics of human IIM and may represent a tool for pre-clinical studies.

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FRI0422 ROLE OF THE PROLYL 3-HYDROXYLASE LEPREL1 IN FIBROSIS

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Background: Three prolyl 3-hydroxylase enzymes, *LEPRE1*, *LEPREL1* and *LEPREL2*, are known to modify prolines in certain sequences in the C-terminal helical region of the polypeptide chains of procollagens converting them to 3-hydroxyproline residues. This modification appears to facilitate correct alignment of the chains in forming the triple helical domains of the procollagen molecules prior to secretion. Increased deposition of triple helical collagen and other extracellular matrix (ECM) proteins by activated fibroblasts underlies pathological fibrosis in systemic sclerosis (SSc), and may be dependent on prolyl 3-hydroxylase activity as a rate-limiting step.

Objectives: The objectives of this study were: 1) to screen candidate genes with large first introns containing regulatory elements for association with systemic sclerosis (SSc) through copy number variation (CNV), 2) to study the lead candidate gene *LEPREL1* further, as a fibrosis-related factor in genetically modified mice subject to bleomycin induced skin fibrosis, 3) to determine the levels of

LEPREL1 protein in SSc and control fibroblasts and measure possible induction by profibrotic factors, and 4) measure the effect of *LEPREL1* gene editing on triple helical collagen secretion by SSc disease fibroblasts.

Methods: DNA samples from SSc patients and controls were assayed by qPCR for CNVs within regulatory regions of 40 candidate genes. The lead candidate, *LEPREL1* was studied further through tagging SNP analysis, of rs7612998, rs1447936, s1018343, s696065, in 564 SSc and 627 controls. In wild type (WT), GPVI α -single knockout (SKO) or *LEPREL1 GPVI α -double knockout (DKO) mice skin fibrosis was initiated by daily subcutaneous bleomycin for 14 days (*LEPREL1 α - embryonically lethal due to placental thrombosis). Skin fibrotic lesions sampled on day 21 were analysed by histology and picrosirius red stain. SSc and control skin fibroblasts grown from forearm skin biopsies, were cultured at passage 3–5, with or without the pro-fibrotic TGF β , or with or without estradiol in cells grown in oestrogen depleted conditions (no phenol red). *LEPREL1* was assayed by Western blot. CRISPR/Cas9 was used to edit the *LEPREL1* gene in SSc skin fibroblasts.**

Results: Initial screening of candidate genes revealed a weak statistical association between a first intron CNV ns514192 and SSc susceptibility in males ($p < 0.028$). However SNP analysis demonstrated that a haplotype, identified as CTAA across the 4 SNPs, was associated with increased risk of SSc development (OR 3.45, $p < 0.0023$). This allele opens a FOXA1 site in the first intron and was seen predominantly in females. In cultured dermal fibroblasts *LEPREL1* protein levels were raised in SSc samples and induced to SSc levels in control fibroblasts by culture with TGF β . Estradiol also induced *LEPREL1* and collagen I protein. In genetically modified *LEPREL1* knockout mice, resistance to bleomycin skin fibrosis was seen (figure 1). Silencing of *LEPREL1* in SSc fibroblasts by CRISPR/Cas9 preferentially reduced triple helical collagen I secretion.

Conclusions: The prolyl 3-hydroxylase *LEPREL1* is confirmed as a fibrosis-related gene associated with SSc susceptibility. Small molecule inhibitors of this endoplasmic reticulum enzyme may limit the overproduction of collagen as a therapy for fibrosis.

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FRI0423

EFFECTS OF SELEXIPAG IN CULTURED SCLERODERMA SKIN FIBROBLASTS

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Background: In systemic sclerosis (SSc), the transition of fibroblasts into profibrotic myofibroblasts contributes to fibrosis through their over-production of extracellular matrix (ECM) proteins, primarily type I collagen (COL-1) and fibronectin (FN), a process which is mediated by the activation of fibrogenic intracellular signalling transduction molecules, including Erk1/2 and Akt kinases.^{1,2} Selexipag and its active metabolite (ACT-333679) are prostacyclin receptor agonists which leads to vasodilation in the pulmonary circulation and mainly used in the treatment of pulmonary arterial hypertension.

Objectives: To investigate the possible effects of selexipag and ACT-333679 in downregulating the profibrotic activity of cultured SSc fibroblasts/myofibroblasts and the fibrogenic signalling molecules involved in the process.

Methods: After obtaining Ethics Committee (EC) approval and informed consent, fibroblasts isolated from skin biopsies of 6 SSc patients (mean age 63±13 years) were cultured until the 3rd culture passage and then either maintained in normal growth medium (untreated cells) or independently treated with different

concentrations of selexipag (from 30 μM to 0.3 μM) or ACT-333679 (from 10 μM to 0.1 μM) for 48 hours. Protein and gene expressions of α -smooth muscle actin (α -SMA), fibroblast specific protein-1 (S100A4), COL-1, and FN were investigated by Western blotting and qRT-PCR.

Erk1/2 and Akt phosphorylation was investigated in untreated and ACT-333679 treated cells at 15 and 30 min as well as at 48 hours by Western blotting.

Results: Selexipag and ACT-333679 significantly reduced the protein synthesis and the gene expression of α -SMA, S100A4, and COL-1 in cultured SSc fibroblasts/myofibroblasts compared to untreated cells, primarily at the concentrations of 3 μM and 0.3 μM for selexipag and 1 μM and 0.1 μM for ACT-333679 ($p < 0.05$). Differently, FN was significantly downregulated at protein level ($p < 0.05$ vs. untreated cells).

Of note, ACT-333679 significantly reduced the phosphorylation of Erk1/2 and Akt kinases already after 30 min of treatment in cultured SSc fibroblasts/myofibroblasts, and this effect was further maintained at 48 hours from treatment ($p < 0.05$ vs. untreated cells, for all tested concentrations).

Conclusions: For the first time selexipag and mainly its active metabolite ACT-333679 were found to interfere with the profibrotic activity of cultured SSc fibroblasts/myofibroblasts, possibly through the downregulation of fibrogenic Erk1/2 and Akt intracellular signalling molecules.

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FRI0424

COMPARATIVE ANALYSIS OF CLINICALLY AFFECTED AND UNAFFECTED SKIN BIOPSIES FROM SCLERODERMA PATIENTS BASED ON RNA-SEQUENCING

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Background: Systemic sclerosis (SSc) is a complex chronic autoimmune disease characterised by vasculopathy, inflammation and fibrosis across different organ systems. The origin and pathogenesis of the disease are not well understood and symptoms may vary among affected individuals, complicating the diagnosis and therapy.

Objectives: We aimed at characterising gene expression profiles in biopsies from affected and unaffected skin of SSc patients.

Methods: RNA isolated from affected and unaffected skin biopsies of 23 SSc patients was analysed using polyA-selected RNA sequencing. Paired differential expression analysis was performed with DESeq2, and Molecular Signatures Database gene set collections were used for functional annotation.

Results: Paired analysis of SSc gene expression profiles identified over 900 genes differentially regulated between the affected and unaffected skin biopsy samples (adjusted p value < 0.05). Macroscopically unaffected skin samples showed clear gene expression differences from the affected skin samples, even though the histological examination showed that pathological changes were already present in most of the samples classified as unaffected. Clustering of differentially expressed genes and subsequent functional characterisation of the resulting gene modules revealed sets of co-expressed genes involved in inflammatory response, extracellular matrix interaction, metabolic function and epithelial differentiation, and highlighted the importance of a number of transcription drivers, such as serum response factor (SRF) or interferon regulatory factor 7 (IRF7). Several genes from the HOX family of transcription factors were found to be among the most significantly differentially expressed genes, pointing to an important role of wound healing and regeneration processes in SSc skin lesions. Additionally, we found significant association between expression of several proteasome subunits and the inflammation score obtained by histological assessment of the skin biopsies.

Conclusions: RNA sequencing analysis in paired skin biopsy samples revealed coordinated changes in the gene expression between SSc lesions and macroscopically unaffected skin, providing insights into the SSc skin pathogenesis at the molecular level.