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* α -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 1, 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 nsv514192 and SSc susceptibility in males ($p < 0.028$). However SNP analysis demonstrated that a haplotype, identified as CTA 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 1. 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.

Disclosure of Interest: None declared

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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.

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Scleroderma, myositis and related syndromes

FRI0425 ASSOCIATION BETWEEN TISSUE OXYGEN DELIVERY AND DIGITAL ULCERS IN SYSTEMIC SCLEROSIS

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Background: Digital ulcers (DUs) are common manifestations of vascular disease in systemic sclerosis (SSc) patients.

Objectives: To investigate the association between the amount of oxygen delivered to peripheral tissues and the development of DUs in SSc patients.

Methods: One hundred and eleven SSc patients (103 female) were recruited consecutively to the study. Thirty age and sex matched controls were also enrolled. Transthoracic echocardiography (TTE) was performed for each patient to estimate cardiac output (CO). Arterial oxygen saturation (SaO₂) with pulse oximeter in room temperature and serum haemoglobin concentration (Hb) were measured at the time of TTE. The amount of oxygen delivered to tissues (D02) in 1 min was estimated using the equation $D02 = CO \times (SaO_2 \times 1.39 \times Hb)$ and adjusted for body weight. Demographic, clinical and laboratory data were retrieved from hospital records. DUs were described as ulcerations on the skin distal to proximal interphalangeal joints (PIF) of the digits. Patients were divided into 2 groups according to presence of DUs within 1 year of study enrollment (Group1=with DU, Group2=without DU). Chi-square and Mann-Whitney U tests were used for comparing categorical and continuous variables between the groups, respectively.

Results: Mean age was 52.6 (11.8) years in SSc patients and 51.3 (13.0) in controls. Median disease duration was 138.0 (1.8–415.2) months. Diffuse disease was present in 30.6% of patients. There were no differences in terms of Hb level, left ventricular ejection fraction (LVEF) and calculated D02 between SSc patients and controls. Mean CO was higher in healthy controls (6.1 vs. 5.1, $p=0.004$). Interestingly, mean S02 measurements was lower in control group (96.8 vs. 98.0, $p=0.005$). Comparison of demographics, disease characteristics, tissue oxygen delivery and relevant variables were summarised in table 1. When patients with active DUs were compared with patients without active DUs; no difference was found in Hb, S02, CO and D02 between groups.

Abstract FRI0425 – Table 1. Comparison of demographics, disease characteristics and D02 parameters between patients with and without DUs

	Group 1 (n=64)	Group 2 (n=47)	p
Age, years (SD)	52.3 (12.0)	49.9 (14.4)	0.34
Disease duration, months (SD)	141.0 (96.8)	177.3 (97.4)	0.06
Female, n (%)	58 (90.6)	44 (93.6)	0.56
Diffuse disease, n (%)	11 (17.2)	23 (48.9)	<0.01
ILD, n (%)	32 (50.0)	35 (74.5)	0.01
PAH, n (%)	3 (4.7)	1 (2.1)	0.63
Ever smoked, n (%)	16 (25.0)	13 (27.6)	0.82
HT, n (%)	15 (23.4)	6 (12.7)	0.22
DM, n (%)	12 (18.7)	3 (6.3)	0.09
Cardiac involvement, n (%)	1 (1.5)	1 (2.1)	1.0
Hb, g/dL	12.8 (1.4)	12.2 (1.4)	0.04
S02, % (SD)	97.8 (2.5)	98.3 (1.1)	0.13
CO, ml/min (SD)	5.1 (1.5)	5.1 (1.6)	0.91
D02, ml/min/kg (SD)	13.6 (4.5)	14.3 (4.6)	0.49

Conclusions: Although the haemoglobin concentration was higher in the non ulcer group, there was no difference in the amount of delivered oxygen between patients with and without DUs. These findings imply that vasculopathy and/or peripheral artery disease plays a major role in the development of ulcers.

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FRI0426

RESPONSE OF THE REACTIVE SPECIES INTERACTOME TO HYPOTHERMIA INDUCED RAYNAUD'S ATTACK IN PRIMARY RAYNAUD AND SYSTEMIC SCLEROSIS

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Background: Although the exact etiology of systemic sclerosis (SSc) remains unknown, a current hypothesis is that reactive species (including reactive oxygen species (ROS), reactive nitrogen species (RNS and reactive sulfur species (RSS)) play an important role in the pathogenesis. In addition, the chemical interactions between these molecules, also known as the reactive species interactome, may lead to the formation of more harmful free radicals, thus leading to more damage. We have previously shown a rapid free thiol increase in SSc patients during a cooling and recovery experiment, however the implications of RNS and RSS in this process remain uncharacterized.

Objectives: The aim of the current study was to explore nitric oxide and sulfate response, as a measure of the unified reactive species interactome, during hypothermia induced Raynaud's attack in systemic sclerosis (SSc), primary Raynaud's phenomenon (PRP) and healthy controls (HC).

Methods: Healthy controls (n=10), PRP patients (n=10) and SSc patients (n=10), all aged ≥ 18 years, were included. All SSc patients fulfilled the ACR/EULAR 2013 criteria. Fingertip photoelectric plethysmography was performed during a standardised cooling and recovery experiment. Patient characteristics were obtained and blood was drawn at four predetermined time points (T0, T1, T2 and T3). In addition to the previously mentioned free thiols, the following markers were also measured: nitros(yl)ated species, nitrite, nitrate, sulfate and the vascular leakage related molecules angiopoietin-1 and angiopoietin-2).

Results: Our results revealed a significantly longer median duration of hypoperfusion and longer recovery time in SSc patients compared to PRP patients and HC. This coincided with stable levels of angiopoietin-1/angiopoietin-2 throughout the experiment. In regards to the thiol modification related molecules, (nitrate, nitrite and RXNO) an increased median level of nitrate (T2: median 29.6 μmol, range 21.99–41.70 μmol, $p=0.041$) was found in SSc patients. Correspondingly, the same trend was observed for the median plasma concentrations of sulfate ($p>0.05$ at all time points).

Conclusions: We have previously shown an increase of free thiols in all patients during the reperfusion phase. This finding sheds new light on the possibility that processes related to oxidative thiol modification, are rapidly reversible. The current findings revealed also a trend in increased levels of nitrate and sulfate in SSc patients. Therefore, it is plausible that these markers of the redox system interact with one another through the same pathway, underlining the complexity of the redox interactome and suggesting potential novel approaches for therapeutic intervention.

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