**Methods:** We enrolled SSc patients, all classified according to the ACR-EULAR criteria. Videocapillaroscopy (NCV) was performed at the time of serum collection. Lung involvement was assessed by spirometry and high-resolution chest CT. Patients were classified into 3 subgroups (no ILD, limited ILD, and extensive ILD) according to the diagram described by Goh et al.[2]. SSC disease activity was assessed according to the activity indices defined by the EUSTAR score.[3] Evaluation of BAG3 protein in serum samples was performed by a sandwich ELISA.

**Results:** The study cohort included 106 SSc patients (47 were classified with lcSSc and 59 with dcSSc) and 100 sex and age matched healthy controls (HC). Serum levels of BAG3 were significantly higher in SSC patients (mean value 85.3 pg/mL, 95% confidence interval CI 47.2-123.4) when compared with HC (0.68 pg/mL, 95% CI 0.13-1.23) (p<0.001). When analyzed according to disease subset, dcSSc patients showed values (143.3 pg/mL, 95% CI 78-208.5) significantly higher and lcSSc patients (8.7 pg/mL, 95% CI 1.64-15.9) (p<0.001). No correlation was found between BAG3 levels and digital ulcers, mRSS and disease activity. Conversely, BAG3 values positively correlated with the extent of lung damage (237.6 pg/mL, 95% CI 131.2-344 in the extensive lung disease vs. 16.3 pg/mL 7.5-25.3 in the limited). Finally, BAG3 values were significantly higher in patients with late NVC pattern in comparison with NVC pattern early/active (p=0.0008).

**Conclusion:** Recent studies have highlighted a central role of extracellular BAG3 in maintaining the tumour microenvironment, as well as in the development of fibrosis in neoplastic tissues. To our knowledge, the presence of BAG3 in the serum of patients with SSC has never been described in the literature. Serum levels of BAG3 were found to be significantly higher in the dcSSc, mostly in those with lung involvement. This is not surprising since the diffuse form of disease has more extensive fibrosis, both in the skin and lung, than lcSSc. Accordingly, BAG3 values correlated with the late pattern at NVC, the one most frequently associated with the more advanced and fibrotic stages of the disease. Conversely, serum BAG3 values did not correlate with disease activity, since these scores reflect the evolution and progression of disease rather than the extent of fibrosis. Indeed, the close correlation between BAG3 levels and the more fibrotic features of the disease, suggests that BAG3 might be a new promising marker of fibrosis.

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**Disclosure of Interests:**

**CONFLICT OF INTEREST:**

**Grants:** The study was conducted without any financial support.

**Funding:** This study was presented in abstract form at the 2023 EULAR Congress.

**Objective:** Our aim was to evaluate the presence of BAG3 in the serum of patients with SSC and to investigate whether circulating levels of BAG3 could have any relationship with different SSC subsets and disease features.

**Objectives:** To study the role of healthy donor- and SSC patient-derived macrophages in the functions of 3 dimensional (D) human cardiac MT fibrosis model.

**Methods:** CD14+ blood-derived monocytes from healthy controls (HC) and SSC patients were isolated and differentiated into human monocyte-derived macrophages (hMMD) using human monocyte colony-stimulating factor (hM-CSF). A timeline of macrophage differentiation stages was performed by incubation of the CD14+ cells for 1,3.5 and 7 days (d) with hM-CSF. One MT was generated with 4’000 human induced pluripotent stem cell-derived cardiomyocytes (ICM), 1’000 human cardiac fibroblasts (HCF) and 1’000 HC or SSc hMMD, mixed in a ratio of 4:1:1 (HC:ICM:HCF-MT or SSc-hMMD:ICM:HCF-MT), and formed by self-assembly in plates with ultra-low-attachment surface. Control MTs were compared with ICM/HCF in a ratio of 4:1:2 (HCF-MT). Two days after MT assembly, the fibrotic condition was induced with transforming growth factor β1 (TGF-β1) for 10d. Contraction of each MT was assessed by recording videos, applying MUSCLEMOTION[2] and subsequent evaluation by relaxation time (ms), contraction amplitude (a.u.), contraction duration (ms), time-to-peak (ms) and beating rate (bpm). The level of fibrosis was analyzed by analysis of collagen levels by mRNA by qPCR, ELISA to measure secreted pro-collagen-I, immunohistochemistry (IHC) using Sirius Red to visualize collagen depositions and degree of fibrosis. Apoptosis was assessed using Caspase-3/7 assay.

**Results:** Control-I CM:HCF-MTs (n=23-49), following TGF-β1 stimulation, revealed a shorter contraction duration (p<0.01) and a lower contraction amplitude (p<0.0001) but not altered contraction duration (p=0.5096) and contraction amplitude (p=0.7296) compared to untreated-I CM:HCF-MTs. Interestingly, untreated-SSc-hMMD:ICM:HCF-MTs showed a shorter time-to-peak (p<0.0001), lower beating rate (p<0.01) and higher contraction amplitude (p<0.01) compared to untreated-HC:ICM:HCF-MTs (n=41-81, both conditions 3d). Next, TGF-β1-stimulated SSc-hMMD:ICM:HCF-MTs exhibited a lower contraction duration (p<0.01) and contraction amplitude (p<0.0001), shorter relaxation time (p<0.0001), and higher beating rate (p<0.0001) compared to TGF-β1-stimulated HC-hMMD:ICM:HCF-MTs (n=33-42, both conditions 1d). Upon TGF-β1-stimulation, control-I CM:HCF-MTs showed significantly increased profibrotic genes: ACTA2 (p<0.001) and COL1A1 (p<0.01) (n=4-5), secreted human pro-collagen-I (n=9-10, p<0.0001), and formed visible collagen-rich fibrotic ring. Remarkably, under fibrotic conditions, addition of HC or SSc hMMD into ICM:HCF-MTs resulted in lower mRNA expression of ACTA2 (n=4-5, p<0.05, both conditions 7d) and COL1A1 (n=4-5, p<0.05, both conditions 7d), lower excretion of human pro-collagen-I protein (n=9-10, p<0.0001, both conditions 7d) and lack of collagen-rich fibrotic ring compared to control-I CM:HCF-MTs. Importantly, independently of culture duration of HC or SSc hMMD, before MT assembly, a similar reduction in profibrotic gene and protein expression was observed. Lastly, the level of apoptosis was similar in all three untreated or TGF-β1-stimulated MTs types.

**Conclusion:** For the first time, the combination of ICM, HCF and hMMD in a 3D fibroblastic cardiac MT model has been established and investigated. Under fibrotic conditions, addition of HC- but not SSc-hMMD into ICM:HCF-MTs influenced MT contractility. Importantly, both HC- and SSc-hMMD reduced TGF-β1-induced fibrotic responses.

**Scheme 1:** Graphical abstract

**REFERENCES:**


**Scheme 1:** Graphical abstract

**DISCLOSURE OF INTERESTS:** Laila Provenzale: None declared, Amelia Hukara: None declared, levgenia Kocherova: None declared, Elena Pachera: None declared, Andrea Laimbachner: None declared, Oliver Distler Speakers bureau: Oliver Distler has had relationships with the following companies in the area of potential treatments for systemic sclerosis and its complications in the last three calendar years: Bayer, Boehringer Ingelheim, Janssen, Medscape. Consultant of: Oliver Distler has had relationships with the following companies in the area of potential treatments for systemic sclerosis and its complications in the last three calendar years: 4P-Pharma, Abbvie, Acceleron, Alcimed, Altavant Siences, Amean, AnaMar, Arxx, AstraZeneca, Baecon, Blade, Bayer, Boehringer Ingelheim, Corbus, CSL Behring, Galapagos, Glenmark, Horizon, Inventiva, Kymera, Lupin, Milltenyi Biotec, Mitsubishi Tanabe, MSD, Novartis, Prometheus, Redpharma, Roivant, Sanofi and Topadur. Grant/research support from: Oliver Distler has...
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POS0626 PERTURBED LIPID METABOLISM IS A CENTRAL METABOLIC REPROGRAMMING HUB IN SYSTEMIC SCLEROSIS

Keywords: Systemic sclerosis, -omics, Skin

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Background: Perturbed cellular metabolism has been increasingly associated with fibroblast activation in fibrosis. A deeper understanding of metabolic rewiring might help to unravel the interplay between the metabolic and fibrotic pathways in systemic sclerosis (SSc).

Objectives: We aimed to identify transcriptomic alterations in metabolic pathway-related genes in fibrotic SSc skin and unravel whether fibroblasts could contribute to the perturbed skin metabolic networks in SSc.

Methods: We integrated transcriptomic microarray data from skin (clinically affected forearm and non-affected back skin) of 76 SSc patients and 26 healthy controls (HC, forearm skin) from three distinct cohorts (GSE:45485, 59785, 9285/32413). Differentially expressed (DE) genes (FDR<0.05) between healthy and SSc skin were identified using the limma package. The supervised gene set enrichment analysis (GSEA) was based on DE genes using the clusterProfiler package, focusing on metabolic pathways. Transcriptional changes in fibroblasts were determined by comparing normal vs TGFβ-stimulated fibroblasts. Differentially expressed (DE) gene expression. Downregulated GO biological processes in TGFβ-treated cultured skin fibroblasts were measured by RNA-seq and scRNA-seq. For these experiments, cultured healthy skin fibroblasts were treated or not with TGFβ for 24h.

Results: Pathway enrichment analysis of DE gene in skin transcripts identified multiple alterations of metabolic pathways in SSc skin (Figure 1A) compared to healthy skin, pointing to enhanced pyrimidine/folate metabolism and suppressed lipid metabolism in SSc skin. Steroid hormone biosynthesis (AKR1, Dhrs1, Cyp1a2, Sult2a1, Hsd1, Ehtm2, Hsd11b2), fatty acid synthesis (fasn1, Fads1, Fads2, ElovL, Scd5, PtpLb) and fatty acid degradation (acat1/2, Acdas, Acaai1/2, Acadm, Acsl3) were the main downregulated lipid metabolism pathways in SSc skin, particularly in patients with the inflammatory intrinsic gene expression subset. The latter changes were detected in the affected and non-affected SSc skin, suggesting that altered lipid metabolism is a generalized feature of the SSc skin. Furthermore, pathway enrichment analysis of TGFβ-induced transcriptional changes in skin fibroblasts, as detected by RNA-seq and scRNA-seq, suggested that TGFβ-driven reprogramming could significantly contribute to the lipid metabolism perturbations in SSc skin (Figure 1B, C). Specifically, STRING analysis revealed that TGFβ suppressed metabolic networks of glucosolphospholipids, arachidonic acid, and fatty acids in cultured skin fibroblasts (Figure 1D).

Conclusion: Our data suggest perturbed lipid metabolic networks in SSc skin and identify skin fibroblasts, exposed to profibrotic milieus, as likely contributors to the altered lipid metabolism in SSc. These results might pave the way to a deeper understanding of the interplay between the metabolic and fibrotic pathways in SSc. Integrating these data with future metabolomic and single-cell studies could accelerate the discovery of potential metabolic targets in SSc.

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POS0627 BLOOD-BASED PROTEIN BIOMARKERS ARE ASSOCIATED WITH SUBCLINICAL CARDIOVASCULAR ABNORMALITIES AS DEFINED BY CARDIOVASCULAR MAGNETIC RESONANCE IMAGING IN SYSTEMIC SCLEROSIS (SSC) PATIENTS

Keywords: Biomarkers, Systemic sclerosis, Cardiovascular disease

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Background: Systemic sclerosis-primary heart involvement (SSc-pH) accounts for up to one-third of SSc-related deaths and clinically apparent pH portends poor outcome. Early detection of SSc-pH is therefore crucial. We have previously shown cardiovascular magnetic resonance (CMR)-detected subclinical myocardial abnormalities. Identifying robust blood biomarkers of SSc-pH would facilitate diagnostic testing, help to resolve the biological mechanisms underpinning SSc-pH and potentially identify new targets for drug development.

Objectives: To identify protein biomarkers associated with subclinical cardiovascular abnormalities as defined by CMR measures in SSc patients, and predominantly inflammatory and cardiometabolic pathways implicated.