Figure 1. Pathways linked to cell metabolism were overrepresented among 3-month upregulated and 6-month lupus mice (F1) downregulated DEGs (differentially expressed genes) compared to controls (C57BL/6).

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Systemic sclerosis, myositis and related syndromes - aetiology, pathogenesis and animal models

POS0422 PANNICULITIS AS A MANIFESTATION OF AN AUTOIMMUNE/AUTOINFLAMMATORY DISEASE

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Background: ASIA syndrome or Schonfeld syndrome is an autoimmune/inflammatory condition induced by adjuvants in genetically susceptible individuals with the development of rheumatic diseases (RD), multiple sclerosis, sarcoidosis, and others.

Objectives: to characterize the symptoms of panniculitis (Pn) associated with ASIA-syndrome

Methods: Within 7 years, ASIA syndrome was diagnosed in 12 women, average age 37.2±7.4, with a referral diagnosis of "Erythema Nodosum" or "Panniculitis". The duration of the disease was 16.7±3.9 months. In addition to general clinical study, the serum concentration of α -1 antitrypsin, amylase, lipase, ferritin, creatine phosphokinase (CPK), immunological parameters (ANP-Hep2, dsDNA, C3 and C4, CRP, ANCA, Scl-70, antibodies to cardiolipins G and M), computed tomography of the chest organs, pathomorphological and immunohistochemical examination of a biopsy specimen of skin with subcutaneous fat tissue (SFA) from the affected areas.

Results: The development of ASIA syndrome was preceded by gluteoplasty using propantriol-1,2,3 (in 4 patients), liposuction (2), biorevitalization with hyaluronic acid (2) and endoprosthetics with breast implants (2), bone metal construction (1) and mesh hernioplasty (1). The clinical picture of Pn was characterized by generalized red-purple painful (VAS pain 55.8±17.3 mm) subcutaneous indurations on the upper limbs and trunk (in 100% of cases), face (16.6%) and lower limbs (33.3%), with ulceration and oily fluid leakage (58.6%). The saucer symptom was recorded in 83.3% of the observed patients. In 66.6% of cases, fever and articular syndrome were observed, in 41.6% – myasthenic syndrome and lymphadenopathy, in 8.3% – xerophthalmia, keratoconjunctivitis dry, xerostomia and recurrent parotitis. In blood tests, leukopenia (up to 2.0x10⁹/l) was identified in 33.3% of patients, a significant increase in the level of creatinine phosphokinase – in 16.6%, a two or more times increase in ESR and CRP – in 100%. All patients demonstrated immunological changes. The morphological picture of the skin and SFA in 75% of cases resembled lobular Pn, in 8.3% – tumor lymphocytes with the immunophenotype of cytotoxic T-lymphocytes: CD3+, CD8+ were detected. The results obtained allowed confirming RD in 8 patients: systemic lupus erythematosus and idiopathic lobular panniculitis in 2 patients (respectively) and 1 in dermatomyositis, systemic scleroderma of

Sjogren's disease, mixed connective tissue disease. One case was diagnosed with sarcoidosis and T-cell lymphoma. In 2 patients after the liposuction procedure the course of Pn was reversible.

Conclusion: Diagnosis of Pn associated with ASIA syndrome is a complex task that requires a multidisciplinary approach to verify the diagnosis and treatment tactics.

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POS0423 NCOA3 AMPLIFIES PROFIBROTIC TRANSCRIPTIONAL PROGRAMS IN SYSTEMIC SCLEROSIS

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Background: Excessive activation of fibroblasts with a TGF β -biased gene signature and deposition of extracellular matrix are key features of fibrotic diseases. The mechanisms underlying these transcriptional changes remain poorly understood. Deregulation, mutations and malfunctions of transcriptional co-regulators, which can interact with multiple transcription factors and enable a broad-spectrum regulation of transcriptional networks, have been implicated as driving factors in a large number of diseases and pathologies.

Objectives: In the present study, we aimed to analyze the role of the co-regulator Nuclear Receptor Co-Activator 3 (NCOA3) in fibroblast activation and tissue fibrosis, and to evaluate a potential interaction of NCOA3 with fibrosis-relevant transcription factors.

Methods: NCOA3 was inhibited genetically by siRNA transfection and pharmacologically by the SRC3 inhibitor-2 (SI-2). We performed bulk RNASeq of human dermal fibroblasts and *in silico* transcription factor binding site screening of differentially expressed genes (DEGs). The interaction of NCOA3 and TGF β -SMAD signaling was analyzed by reporter and CoIP assays.

Results: The expression of NCOA3 in skin biopsies of SSc patients compared to normal controls demonstrated that SSc fibroblasts express modestly, but significantly reduced levels of NCOA3, which persisted in cultured SSc fibroblasts. Stimulation of normal fibroblasts with chronically high levels of TGF β as they also occur in fibrotic tissue remodeling strongly decreased NCOA3 expression to a similar extent as in SSc fibroblasts. Furthermore, NCOA3 expression is also deregulated in different murine models of skin fibrosis. To investigate the functional effects of decreased NCOA3 levels, we targeted the expression of NCOA3 in normal fibroblasts. siRNA-mediated knockdown of NCOA3 ameliorated TGF β -induced gene expression, collagen release, myofibroblast differentiation and cell proliferation. In contrast, knockdown of NCOA3 had no effects on collagen release, expression of contractile proteins or gene expression in unstimulated fibroblasts, suggesting that NCOA3 is not required for cellular homeostasis. To characterize the molecular mechanisms, we performed RNASeq upon NCOA3 knockdown. We identified 343 significant differentially expressed genes (220 downregulated and 123 upregulated with a Benjamini-Hochberg false discovery rate FDR < 0.25 and fold change > 1.5) between TGF β -stimulated fibroblasts with and without NCOA3 knockdown (NCOA3-DEGs) including the fibrosis-relevant genes *EDNRB*, *COL5A3*, *HES1*, *IL11* or *IL33*. Functional analysis of the NCOA3-DEGs showed enrichment of pathway terms such as collagen binding and extracellular matrix organization. *In silico* screening of the promoters of the NCOA3-DEGs for potential transcription factor binding motifs revealed binding motifs of core transcription factors for fibroblast activation and tissue fibrosis such as SMAD2/3/4, RBPJ, ZEB1, TCF4, REL, and SNAIL2 amongst the downregulated NCOA3-DEGs. Experimental validation of our bioinformatic results using SMAD3 as example demonstrated a higher percentage of NCOA3-pSMAD3 double-positive fibroblasts in skin sections of SSc patients compared to healthy controls. In addition, knockdown of NCOA3 reduced TGF β -induced SMAD-reporter activity. Furthermore, stimulation with TGF β increased the interaction of NCOA3 with SMAD3 as analyzed by co-immunoprecipitation. Simultaneous knockdown of NCOA3 and SMAD3 showed no additional reductions compared to the single knockdowns, suggesting that NCOA3 controls SMAD3-dependent gene transcription under fibrotic conditions. Finally, inhibition of NCOA3 showed anti-fibrotic effects in different murine models of experimental skin and lung fibrosis.

Conclusion: Our findings characterize NCOA3 as regulator of multiple pro-fibrotic transcription programs. Pharmaceutical inhibition of NCOA3 might be a strategy to interfere simultaneously with several core pro-fibrotic mediators in fibrotic diseases such as SSc.

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POS0424

DETERMINING CIRCULATING ENDOTHELIAL CELLS USING CELLSEARCH SYSTEM IN SYSTEMIC SCLEROSIS PATIENTS

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Background: Endothelial damage and fibroproliferative vasculopathy of small vessels are pathological hallmarks of Systemic Sclerosis (SSc). Detection and analysis of circulating endothelial cells (CECs) detached from affected blood vessels may be an informative tool to study vascular dysfunction and could be considered a novel biomarker of scleroderma vasculopathy. Our group first showed the presence of CECs in SSc by fluorescence-activated cell sorting (FACS), demonstrating that a raised counts of active CECs may represent direct evidence of active vascular disease in SSc. Despite these interesting data, issues related to difficulties in CEC counting through FACS analysis, due their very low concentration in peripheral blood, prevented further investigations in this field. Recently, a specific kit for the detection of CECs has been developed through the CellSearch System (CS), a semi-automated device for the standardized analysis of rare cells, such as CECs, in peripheral blood.

Objectives: To assess the counts of CECs determined by the CS in SSc patients and to evaluate their clinical implication and potential as vascular biomarker in SSc.

Methods: 10mL of blood samples were collected from 29 subjects (19 SSc patients and 10 healthy donors - HDs) and stored in tubes containing a specific preservative, to allow the analysis of 4mL of blood within 72 hours, according to manufacturer instructions. Out of 19 SSc patients, 18 were female, 10 had the limited form and 9 the diffuse cutaneous variant of SSc. CS uses a proprietary kit containing a ferrofluid-based reagent, that target CD146 to magnetically capture CECs, and the immunofluorescent reagents to stain the CECs, defined as CD146+, CD105-PE+, DAPI+ and CD45-APC-. Clinical, laboratoristic and demographic data were also collected.

Results: The mean number of CECs in patients with SSc was significantly higher in comparison to HDs (554/4mL vs. 53.5/4mL, p=0.0042). When analyzed according to disease subset, both lcSSc and dcSSc showed significantly increased levels of CECs in comparison with HDs (p=0.003 and p=0.005, respectively). No statistical difference was observed in the mean number of CECs in patients with lcSSc compared to those with dcSSc. Regarding vascular involvement, the CECs counts strictly correlated with the presence of digital ulcers (DUs) (p=0.0001) showing a median of 863cells/4mL for the SSc patients with DUs versus a median of 276.2/4mL for the SSc patients without DUs. No statistical correlation was found between CECs and serological autoantibody pattern, skin parameters, or joint and muscle involvement. Patients with active disease, according to the EUSTAR Activity Index, showed a higher CECs value than those with inactive disease (p=0.0012).

Conclusion: The amount of CECs detectable in peripheral blood has been recently proposed as a marker of endothelial damage in different vascular diseases, including SSc. However, currently no standardized method is available to determine CEC counts, which makes reported data on CECs reliable and suitable. The CS system is a commercially available semi-automated system that enables standardized determination of CECs. Thus, we examined clinical utility of CECs count by this system in SSc patients. Our results confirm that baseline CEC counts, evaluated by a new standardized method, may represent direct evidence of endothelial damage in SSc and could be a promising tool for monitoring active disease and evaluating therapeutic responses to vascular and immunosuppressive treatments.

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POS0425

INCREASED KERATIN 9 EXPRESSION IN SYSTEMIC SCLEROSIS SKIN IS DRIVEN BY THE LNCRNA HOTAIR FROM FIBROBLAST DERIVED EXOSOMES

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Background: Skin fibrosis is the hallmark fibrotic manifestation of systemic sclerosis (SSc). Despite a key role of tissue fibroblasts, skin changes extend to the keratinocyte layer, which contribute to the loss of skin function. RNA seq. analysis of SSc patient forearm skin showed that palmo-plantar specific Keratin 9 (CK9) was highly expressed (1). SSc affected skin shares several features with palmo-plantar skin including increased keratinocyte layer thickness and lack of hair. Seminal work of last decade has shown that long noncoding RNA in the HOX loci play a crucial role in skin keratinocyte differentiation (2), with the lncRNA HOTAIR being one of the HOX lncRNA mostly expressed in the palmo-plantar region.

Objectives: Following recent data suggesting a role of HOTAIR in the fibrotic phenotype of dermal fibroblasts in SSc (3), here we set out to determine if HOTAIR expressed in SSc dermal fibroblasts was a contributing factor to the high levels of CK9 found in SSc patient skin

Methods: Full-thickness skin biopsies were surgically obtained from the forearms of patients with SSc of recent onset. Fibroblasts were isolated and cultured in monolayers. HOX transcript antisense RNA (HOTAIR) was expressed in healthy dermal fibroblasts by lentiviral induction employing a vector containing the specific sequence. Exosomes were isolated from dermal fibroblast media using the Total exosome isolation reagent (Thermo Fisher). Enhancer of zeste 2 (EZH2) was blocked with GSK126 inhibitor. Skin equivalents were created using scramble and HOTAIR expressing fibroblasts with primary keratinocytes

Results: Media from both SSc patient fibroblasts and HOTAIR expressing fibroblasts induced CK9 expression in healthy keratinocytes *in vitro*. In addition, HOTAIR expressing fibroblasts induces CK9 expression in keratinocytes in 3D skin equivalent models. Media fractionation studies indicated that HOTAIR was present in fibroblasts exosomes and found at a higher concentration (2.7 fold p=0.01) in exosomes from SSc fibroblasts. Importantly, transfection of Exosomal RNAs from SSc fibroblasts could reproduce the increase in CK9 in keratinocytes. Mechanistically, CK9 induction was mediated by changes to the histone methylation profile in the keratinocytes through EZH2.

Conclusion: Pro-fibrotic dermal fibroblasts in systemic sclerosis contribute to the overall skin loss of function by inducing CK9 in adjacent keratinocytes through transfer of the long non-coding RNA HOTAIR. Unraveling the crosstalk of activated fibroblasts with adjacent cells may lead to identify therapeutic targets to re-establish tissue homeostasis and function during fibrosis.

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POS0426

CIRCULATING MICRORNA PROFILING IN PATIENTS WITH ANTI-SYNTHEASE SYNDROME AND INTERSTITIAL LUNG DISEASE

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Background: Anti-synthetase syndrome (ASSD) is an autoimmune disease characterized by autoantibodies against one of many aminoacyl transfer RNA (tRNA) synthetases. Interstitial Lung Disease (ILD) in ASSD patients is frequent, often severe and rapidly progressive, causing much of the increased morbidity and mortality associated with ASSD as compared to other idiopathic inflammatory myopathies [1].