**Objectives:** In this study, we hypothesized that immune-related miRNAs may be associated with presence/absence of lung involvement in patients with ASSD and help predict disease course.

**Methods:** A total of 15 ASSD patients were enrolled: 11 with ILD and 4 without ILD. Differentially expressed miRNAs were identified in plasma derived exosome-uses, using miRNA PCR array (MHS-111Z, Qiagen) including 84 miRNAs involved in activation and differentiation of T and B cells.

**Results:** Among all miRNAs analyzed we found that miR-15b-5p, miR-23a-3p, miR-25-3p, miR-32a-3p and miR-29c-3p were up-regulated in ASSD-ILD patients (p<0.05) as compared to patients without lung involvement (Figure 1). To evaluate the effectiveness of the five miRNAs for predicting ILD among ASSD patients, ROC curves were constructed. The AUCs of miR-15b-5p, miR-25-3p, miR-30a-5p and miR-29c-3p were 0.83, 0.87, 0.86 and 0.89, respectively (p= 0.05 for miR-25-3p and p<0.05 for all other curves). The prediction of the biologic targets and pathways as well as cellular processes by DIANA-mirPath analysis showed that all miRNAs were associated with ILD presence and involved in PI3K-Akt signaling pathway.

**Conclusion:** Our study shows that, in ASSD patients with ILD, miR-15b-5p, miR-23a-3p, miR-25-3p, miR-30a-5p and miR-29c-3p were up-regulated compared to patients without evidence of ILD. A clear involvement in immune and inflammatory diseases was documented for the miRNAs identified [2] and, for many of these, studies in the literature indicate a possible role in pulmonary fibrosis [3]. It is notable that these miRNAs were related to PI3K-Akt signaling pathway that regulate cell proliferation, differentiation and apoptosis [4]. It has also been demonstrated that in lung fibroblast the PI3K-Akt signals can be aberrantly activated [5]. The identification of markers could be important in the early identification of the disease and for its treatment.

**RESULTS:**

**REFERENCES:**


**Disclosure of Interests:** None declared.

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**POSO427**

**REGULATION OF IFN SIGNATURE BY HDAC CLASS II-CD2S AXIS IN SYSTEMIC SCLEROSIS**

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**Background:** Systemic sclerosis (SSc) is associated with an interferon (IFN) signature, which is defined by a higher expression of IFN-stimulated genes (mainly in response to IFNα). Histone deacetylases (HDACs) are a family of epigenetic modifiers mediating immune function. HDACs function via diverse molecular mechanisms, including direct inhibition of gene transcription or indirectly through modulation of nuclear transcription factors such as NF-kB and ST8S. CD52 protein regulates T cell receptor and NF-kB signalling. Previously, we showed downregulation of CD52 in SSc monocytes, however, the influence of CD52 on IFN up-regulation has not been studied yet.

**Objectives:** We investigated the role of CD52 in the regulation of IFN response in monocytes. Moreover, we explored the regulatory mechanisms of CD52 expression to identify the involvement of HDACs in that process.

**Methods:** RNAseq of CD14+ monocytes isolated from peripheral blood of lcSSc (n=5, age=54.4±6.7), dcSSc patients (n=5, age=51.8±7.2) and age- and sex-matched healthy controls (HC) (n=5, age=50.8±8.7) 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. CD25 activity in monocytes was blocked by monoclonal antibody Alemtuzumab [10ug/ml] and IFN signature genes expression was assessed upon IFNα stimulation [1ng/ml] by qPCR and ELISA. HDAC-dependent regulation of CD52 expression in CD14+ monocytes from HC was analysed on mRNA and protein levels after treatment with pan-HDAC inhibitor valproic acid and HDAC class Ia inhibitor TMP269 (both 2.5μM).

**Results:** Pathway analysis revealed significant alterations in interferon signalling in SSc monocytes. Monocytes treated with Alemtuzumab and IFNα showed induced expression of STAT1 (p=0.023, N=4), CXCL9 (p=0.062, N=4) and CXCL10 (p=0.005, N=4). CD52 expression revealed a downregulation in SSc monocytes with induced expression of HDAC6, 10, 6, 11 (p<0.05) and reduced HDAC1, 3 and 8 (p<0.05). CD52 mRNA was significantly decreased after IFNα stimulation (p=0.001, N=3). Treatment with valproic acid and HDAC class Ia inhibitor TMP269 resulted in decreased phosphorylation of STAT1 (p>0.01, N=4) followed by a declined level of CXCL10 (p=0.01, N=4) and restored level of CD52 mRNA (p=0.001, N=4).

**Conclusion:** Our findings demonstrated a new aspect of pro-inflammatory type I IFN signalling in SSc. We described a novel regulation feedback loop in monocytes, in which CD52 suppresses IFN signature, while its expression is inhibited by IFN-induced HDAC activity (mainly HDAC class Ila). Therefore, targeting the CD52-IFN-HDAC axis might serve as a novel therapeutic strategy in SSc.
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POS0429

INTERLEUKIN-4 ACTIVATES EOSINOPHILS AND CCR3-POSITIVE T HELPER CELLS MIGRATION TO FASCIA AND PROMOTES FIBROSIS IN EOSINOPHILIC FASCITIS

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Background: Eosinophilic fascitis (EF) is a rare disease that causes inflammation and fibrosis mainly in the fascia of the extremities with eosinophilia. It has been reported that the hypertrophied fascia in EF shows inflammatory cell infiltration by the lymphocytes and eosinophils and increased expression of fibroblast-related cytokines genes in fibroblast [1]. However, its pathophysiology in the fascia remains unresolved.

Objectives: Therefore, we focused on fascial fibroblasts and aimed to determine the role of interleukin-4 (IL-4) in eosinophil and helper T cell infiltration and fibrosis in fascial fibroblast in EF.

Methods: Fascial fibroblasts were obtained from fascia biopsy of a patient with EF, and were stimulated with pre- and post-treatment serum of a patient with EF and healthy control, followed by microarray to analyze gene expression. Fascial fibroblasts were stimulated with IL-4 10 ng/mL, and gene expression of IL-4 receptor and CCR3 ligands, CCL7 and CCL11 were measured by qPCR. Transforming growth factor (TGF) -β and perisin in the pre- and post-treatment serum of a patient with EF and conditioned medium of fascial fibroblasts stimulated with IL-4 were measured by ELISA. To examine the role of IL-4 in proliferation, we performed in proliferation assays using fascial fibroblasts treated with IL-4, CCR3-positive T cells in the fascial tissue of EF, dermatomyositis, and polymyositis patients were evaluated by immunostaining.

Results: By microarray analysis, CCL7 and CCL11 expression of fascial fibroblasts stimulated with pre-treatment EF serum was higher than that in post-treatment EF serum and control serum. CCL7 and CCL11 mRNA in IL-4 stimulated fascial fibroblasts were increased in 1.1-fold and 73-fold, respectively. TGF-β and perisin in IL-4 stimulated fascial fibroblast conditioned medium were also increased. In addition, TGF-β and perisin in EF serum were gradually decreased by treatment for 4 and 10 weeks, compared to before treatment. Finally, fascial fibroblast proliferation was significantly increased by stimulation with IL-4. Furthermore, infiltration of CCR3-positive T cells was specific to the fascia tissue of EF.

Conclusion: Eosinophils and eosinophilic fascitis involve abnormal autophagy-related protein and cytokine expression. The expression of TGF-β and perisin in EF serum was significantly decreased in the pre- and post-treatment EF serum. In the future, we will further examine the role of TGF-β and perisin in the pathogenesis of EF.

REFERENCES:


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POS0430

EXPRESSION AND CLINICAL SIGNIFICANCE OF AUTOPLAYOPHAGY-RELATED GENES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF SYSTEMIC SCLEROSIS

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Background: Growing evidences have demonstrated that autophagy is a powerful regulator in the pathogenesis of fibrosis and autoimmune diseases. Auto- phagy abnormalities in SSc involve abnormal autophagy-related protein and autophagy-related gene polymorphism1-5, however, there is a few reports on the expression and clinical significance of autophagy-related genes.

Objectives: To investigate the expression and clinical significance of autophagy-related genes LC-3 mRNA, Becline-1 mRNA, Agt-3 mRNA, Agt-5 mRNA, Agt-12 mRNA and Agt-16L1 mRNA in peripheral blood mononuclear cells (PBMC) of systemic sclerosis (SSc).

Methods: 51 cases of SSc and 60 cases of normal control were received from the Affiliated Hospital of North Sichuan Medical College, and autophagy-related genes were detected by RT-PCR. SPSS19.0 statistical software was used to compare the expression of autophagy-related genes between groups and analyze the relationship between autophagy-related genes and clinical data.

Results: LC-3, Becline-1, and Agt-3 were highly expressed in SSc compared with normal control (LC-3: 0.78(0.60) ×10^-3 vs. 0.52(0.54) ×10^-3; Becline-1: 6.68(3.56)×10^-3 vs. 5.22(3.54)×10^-3; Agt-3: 17.58(12.33)×10^-3 vs. 11.04(5.46)×10^-3, P<0.05), however Agt-5, Agt-12 and Agt-16L1 of autophagy-related genes were not statistically significant (AGT-5: 6.67(3.58)×10^-3 vs. 6.67(2.64)×10^-3; AGT-12: 8.64(5.65)×10^-3 vs. 8.57(4.66)×10^-3; Agt-16L1: 2.69(2.19)×10^-3 vs. 2.52(2.26)×10^-3) (Figure 1). Beclin-1 and Agt-5 high expressed in SSc with the positive of anti-SSA/Ro antibody. LC-3 was positively correlated with Age(r=0.662) and ESR(r=0.355) (all P<0.05).

Conclusion: Autophagy-related genes were increased in PBMC of SSc, and were correlated with Age, ESR and autobody, suggested that autophagy is a key feature in the pathogenesis of systemic sclerosis.

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Figure 1. The relative expression of autophagy-related genes