Background: Systemic Sclerosis (SSc) is one of the rheumatic diseases burdened with obstetrical complications. An Italian multicenter study showed that women with SSc have a higher-than-normal risk of intraperinatal growth restriction, preterm delivery, very-low birth weight babies and pregnancy should be discouraged in patients with severe organ damage. However, with a multidisciplinary management, patients with SSc can have successful outcomes [1]. Little is known about the pathogenesis of obstetrical complications, as studies on placentas are case reports or description of a few cases [2].

Objectives: The aim of this study was to analyze the placental alterations with a focus on the role of inflammation in the pathogenesis of obstetrical complications in SSc, including the study of the atypical chemokine receptor 2 (ACKR2), involved in immune modulation and known to be highly expressed in circulating leukocytes in SSc patients [3].

Methods: Eight SSc pregnant patients were compared with 16 patients with other rheumatic diseases (ORD) and 16 healthy controls (HC), matched for gestational age. Clinical data were collected. Placentas biopsies were obtained for histopathological analysis and immunohistochemistry for CD3, CD20, CD11c, CD68 and ACKR2. Frozen placenta samples from 4 SSc, 8 ORD and 8 HC were analyzed by qPCR for ACKR2 gene expression and proteins were extracted for multiplex assay for cytokines, chemokines and growth factors involved in angiogenesis and inflammation. Statistical analysis was performed with parametric or non-parametric tests depending on samples distribution.

Results: The number of placental CD3 (p<0.05), CD68 (p<0.001) and CD11c+ (p<0.001) cells was significantly higher considering the group of patients affected by rheumatic diseases (SSc+ORD) compared to HC. The SSc group alone did not show significance due to the lower sample size. No differences were found between both groups in terms of vascular alterations or fibrosis. The percentage of stained area for ACKR2 and the ACKR2 transcripts levels were comparable between groups. Hepatocyte growth factor (HGF), involved in angiogenesis, was significantly increased in the group of rheumatic diseases patients (SSc+ORD) compared to HC (p<0.05), while the chemokine CCL5 was significantly higher in SSc patients compared to patients affected by ORD (p<0.05) and to HC (p<0.01). CCL5 levels directly correlated with the number of all inflammatory cells considered and higher levels were associated to histological villitis (p<0.01).

Conclusion: The higher number of placental inflammatory cells and the alterations in the levels of HGF and especially CCL5 could play a role in the pathogenesis of the obstetrical complications in SSc. ACKR2 does not seem involved in the obstetrical complications of SSc.

References:

Disclosure of Interests: None declared

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SOLUBLE GUANYLYL CYCLASE REDUCED THE GASTROINTESTINAL FIBROSIS IN BLEOMYCIN-INDUCED MOUSE MODEL OF SYSTEMIC SCLEROSIS

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Background: Systemic sclerosis (SSc) is a chronic autoimmune-mediated connective tissue disorder. Although the etiology of the disease remains under mined, SSc is characterized by fibrosis and proliferative vascular lesions of the skin and internal organs. SSc involves the gastrointestinal tract in more than 80% of patients. Soluble guanylate cyclase (sGC) is used to treat pulmonary artery hypertension (PAH), and has been shown to inhibit experimental skin fibrosis 2.

Objectives: The aim of this study is to investigate whether bleomycin (BLM)- treated mice show gastrointestinal fibrosis, and find a therapeutic strategy to the lesion.

Methods: Female C57BL/6J mice were treated with BLM or normal saline by subcutaneous implantation of osmotic minipump. These mice were sacrificed on day 28 or day 42. Gastrointestinal pathologies were examined by Masson Trichrome staining. The expression of fibrosis-related genes in gastrointestinal tract were analyzed by real-time PCR, and the levels of collagen in the tissue was measured by Sircol collagen assay. To evaluate peristaltic movement, the small intestinal transport (ITR%) was calculated as [Dyeing distance × (Duodenum- Appendix)] -1 × 100 (%). We treated BLM-treated mice with soluble guanylate cyclase (sGC) or DMSO orally and analyzed them.

Results: Histological examination revealed that fibrosis from lamina propria to muscularis mucosa in the esophagus was significantly increased in BLM-treated mice, and 0.03, and to both icSSc (OR 2.80, 95% CI 1.16 to 6.84, p = 0.02) and dcSSc (OR 3.42, 95% CI 1.20 to 9.72, p = 0.02) subtypes. The Srps55 rs2235611 A minor allele and AA genotype showed a significant risk association with susceptibility to SSc-related pulmonary fibrosis (A allele: OR 1.39, 95% CI 1.00 to 1.93, p = 0.046; AA genotype: OR 3.95, 95% CI 1.48 to 10.54, p = 0.006). A trend toward an association between the AA genotype and anti-Scl70 antibody-positive SSc was also found (OR 2.62, 95% CI 0.95 to 8.37, p = 0.06). Both rs2235611 A allele and AA genotype were significantly associated with the SSc subset without digital ulcers (A allele: OR 1.33, 95% CI 1.01 to 1.75, p = 0.04; AA genotype: OR 3.26, 95% CI 1.32 to 8.03, p = 0.01).

Conclusion: The Srps55 rs2235611 polymorphism is associated with susceptibility to SSc and, in particular, with SSc-related pulmonary fibrosis and peripheral vascular phenotype, consistent with a role of VEGF-A pre-mRNA alternative splicing in the development of pulmonary fibrosis and impairment of angiogenesis. Further replication studies are warranted to confirm our findings in independent SSc cohorts.

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EPIGENETIC DEREGULATION OF AUTOPHAGY PROMOTES FIBROSIS IN SYSTEMIC SCLEROSIS

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Background: Autophagy is a catabolic process allowing cells to degrade unnecessary or dysfunctional cellular organelles. Failure of appropriate regulation of autophagy, however, can severely perturb tissue homeostasis. Several stimuli present in fibrosis such as pro-fibrotic cytokines are known to activate autophagy.

Objectives: The objective of this work was to characterize the regulation of autophagy in systemic sclerosis (SSc) and to decipher its role in the pathogenesis of SSc.

Methods: Activation of autophagy in SSc skin and matched tissue samples from healthy individuals was assessed by immunofluorescence staining for ATG7, BECLIN1 and P62. We generated ATG7−/−Col1a2CreER mice to selectively disable autophagy in fibroblasts. The role of the autophagy was investigated in the model of bleomycin- and TGFβ1/Bleomycin-induced dermal and pulmonary fibrosis. Overexpression of Mst1 was achieved by adenovirus encoding for Mst1. Collagen release and protein expression were measured by Western blot. Target genes were analyzed by RT-PCR. Co-immunoprecipitation and reporter assay were performed to study physical and functional interactions between Mst1 and SMAD3. To monitor the autophagic flux in vitro and in vivo we generated adenoviral vectors encoding for tandem fluorescent-tagged LC3 (mRFP-EGFP-LC3).

Results: Transforming growth factor-β (TGFβ) activates autophagy by an epigenetic mechanism to amplify its profibrotic effects. TGFβ induces autophagy in fibrotic diseases by SMAD3-dependent downregulation of the H4K16 histone acetyltransferase MYST1, which regulates the expression of core components of the autophagy machinery such as ATG7 and BECLIN1. Activation of autophagy in fibroblasts promotes collagen release and is both, sufficient and required, to induce tissue fibrosis. Forced expression of MYST1 abrogates the stimulatory effects of TGFβ on autophagy and re-establishes the epigenetic control of autophagy in fibrotic conditions. Interference with the aberrant activation of autophagy inhibits TGFβ-induced fibroblast activation and ameliorates experimental dermal and pulmonary fibrosis. These findings link uncontrolled TGFβ signaling to aberrant autophagy, deregulated epigenetics in fibrotic diseases and may open new avenues for therapeutic intervention in fibrotic diseases.

Conclusion: We demonstrate that the epigenetic control of autophagy is disturbed by a TGFβ-dependent downregulation of the H4K16 histone acetyltransferase MYST1. The increased activation of autophagy induces fibroblast-to-myofibroblast transition and promotes fibrotic tissue remodeling. Re-expression of MYST1 prevents aberrant autophagy, limits the profibrotic effects of TGFβ and ameliorates experimental fibrosis. Restoration of the