against targets not tested for in clinic. In this study, we aimed at extending the detection of autoantibodies by including all cytoplasmic aaRS in the analysis of patients with IIM. We hypothesized the existence of new potential autoantigens with new aaRS protein signatures.

Methods: The presence of anti-aaRS autoantibodies was determined using a multiplex suspension bead array assay on 242 IIM patients from the Karolinska University Hospital myositis cohort. A panel of 186 recombinant constructs, representing 57 proteins that included full-length or partial sequence overlaps between constructs of all cytoplasmic aaRS as well as other myositis related proteins, were coupled to magnetic color-coded beads and each plasma sample was tested against the complete antigen panel.

Results: By the use of this multiplex method we identified patients with autoantibodies against many of the tested aaRS. Autoantibodies binding to HisRS have previously been shown to bind with higher reactivity to the WHEP domain of HisRS and this was also confirmed in this study. We confirmed reactivity against three of the other aaRS tested for in the clinic (PL-12, PL-7, and EJ). In addition, we identified patients positive for anti-Zo-, KS and HA, autoantibodies usually not screened for in routine. Finally, our data indicates that there are autoantibodies binding to other aaRS than the previously known eight autoantigens, which will be presented.

Conclusion: In this study, we could detect autoantibodies in plasma from patients with IIM, both against the most common aaRS autoantibodies, but also against other aaRS that are usually not tested for in clinic. We conclude that it is important to continue the studies of anti-aaRS autoantibodies, and their correlation to clinical manifestations, and in the long run also include more aaRS autoantigens in clinical practice.

References:

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THE INS AND OUTS OF EPIDERMAL DYSFUNCTION IN SYSTEMIC SCLEROSIS (SSc): RESULTS FROM A NOVEL TISSUE ENGINEERED EPIDERMAL EQUIVALENT FROM SSC KERATINOCYTES

B. Russo1, J. Borowczyk-Michalowska1,2, W. H. Boehncke2, N. Brembilla1,2, C. Chizzolini1. 1Pathology & Immunology School of Medicine, University of Geneva, Geneva, Switzerland; 2Department of Dermatology, University Hospital and School of Medicine, University of Geneva, Geneva, Switzerland

Background: Skin fibrosis is a hallmark of systemic sclerosis (SSc). It is commonly accepted that vascular damage, immune system activation and, abnormal fibroblasts-to-myofibroblasts differentiation are pathological capital features. Nevertheless, recent evidence portrays a potential role of the epidermis in the pathogenesis of SSc skin fibrosis (1, 2). This new angle on skin fibrosis pathogenesis is particularly attractive as the epidermis is an easy to access therapeutic target.

Objectives: To dissect the potential epidermal dysfunction in SSc and its effect on dermis homeostasis, using a novel epidermal equivalent reconstructed from SSc keratinocytes.

Methods: Primary keratinocytes and fibroblasts cell lines were generated from skin biopsies obtained from 6 SSc and 6 healthy donors (HD), upon informed consent and ethical approval. Epidermal equivalents (EE) were generated from 4 SSc and 6 HD keratinocytes. Skin and EE expression of the mitotic marker Ki67, of the differentiation markers (K10, involucrin, filaggrin, loricrin), and activation markers (K6, K18) was evaluated by immunohistochemistry. The transcriptomic profile of SSc keratinocytes in monolayer or stratified in EE was identified by RNAseq analysis. EE conditioned medium was used to stimulate fibroblasts. The fibroblast production of interleukin (IL)-6, IL-8, matrix metalloproteinase (MMP)-1, type-I collagen (col-I), and fibronectin was assessed by ELISA.

Results: Compared to HD, immunohistochemistry revealed that SSc epidermis is characterized by aberrant premature differentiation and enhanced expression of activation markers associated with a lower mitotic rate of basal keratinocytes. Of interest, EE reconstituted from SSc keratinocytes reproduced most of the abnoromalities observed in SSc epidermis. RNAseq analysis revealed that SSc keratinocytes, either cultured in monolayer or in EE, have a distinct transcriptomic profile compared to their HD counterpart, characterized by the downregulation of genes from the HOX family.

The supernatant of EE enhanced the production of IL-6, IL-8, MMP-1, col-I, and fibronectin by HD fibroblasts (p<0.05). Except for col-I and fibronectin, this effect was 2-fold higher in the presence of supernatant from EE reconstituted by SSc keratinocytes. Neutralization experiments indicated that IL-1 was, at least in part, responsible for keratocyte-dependent fibroblasts activation.

Conclusion: We established a novel epidermal equivalent tissue engineered from SSc keratinocytes, that recapitulates the in vivo characteristics of SSc epidermis. Our preliminary data suggest that SSc keratinocytes have an intrinsic altered program of differentiation, possibly due to the downregulation of some HOX genes. This altered phenotype is associated with increased production of mediators that stimulate fibroblasts production of inflammatory cytokines. In this scenario, we may hypothesize that SSc epidermis participates in modifying the dermis environment, favoring the development of chronic inflammation and fibrosis.

References:

Disclosure of Interests: Barbara Russo: None declared, Julia Borowczyk-Michalowska: None declared, Wolf-Henning Boehncke Consultant of: WHB received honoraria as advisor or invited speaker from Abbvie, Almirall, BMS, Celgene, Leo, Lilly, Novartis, UCB, Speakers bureau: WHB received honoraria as advisor or invited speaker from Abbvie, Almirall, BMS, Celgene, Leo, Lilly, Novartis, UCB. Nicolò Brembilla: None declared, Carlo Chizzolin Consultant of: Boehning Engelheim, Roche

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SAT0290 HIGH SERUM MYOSTATIN LEVELS SUGGEST ACCELERATED MUSCLE SENECESSENCE IN ACTIVE IDIOPATHIC INFLAMMATORY MYOSITIS

A. Anuja1, M. Singh1, M.K. Rai1, H. Singh1, V. Agarwal1, L. Gupta1, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

Background: Inflammation is the forerunner to fibrosis and premature ageing in various systemic diseases. Hence it seems plausible that idiopathic inflammatory myopathies (IIM) may exhibit accelerated senescence too.

Objectives: Hence we investigated the Myostatin: Follistatin system in the serum as a reflection of early senescence in myositis as compared with healthy and diseased controls.

Methods: Patients with inflammatory myositis (ACR/EULAR criteria) presenting to the wards and outpatient clinic between December 2017 to August 2019 were recruited. Those with active infection, pregnancy, renal dysfunction or chronic kidney disease were excluded. Apart from patient and disease variables, activity and damage were assessed using standard IMACS score set measures. Patients in inception cohort were additionally followed up at 1 and 6 months. Myostatin and follistatin were estimated in sera using ELISA (R&D systems, USA). Juvenile myositis and young adults (18-40 years) were subsequently analyzed separately. Non-parametric tests were used for paired and unpaired analysis. Results expressed as median.

Results: 95 myositis (8 Juvenile myositis, 26 DM, 10 PM, 29 Overlap, 2 NAM 1 CAM and 19 ASS) patients (23 Male and 72 Female) with median age 38 (24.5-46.0) years and disease duration 0.9 (2.3-5.1) years were included. Serum Myostatin was lower in IIM than in healthy control (HC) (153.5 vs 243.6 p<0.001, Fig 1A) but higher in IIM as compared with disease controls (153.5 vs 86.1 p=0.0174 Fig 1B). Serum myostatin was comparable between juvenile and adult myositis and in the various subsets of adult myositis (Fig 1 C and D). Myostatin levels were higher in active as compared with inactive myositis in young adults (211.7 vs. 158.9, p=0.0419, Figure 1E). Serum Myostatin correlated with height (r 0.3, p=0.003) and weight (r 0.2, p=0.047) but not MMT8 or muscle enzymes.
Although Follistatin was lower in IIM than HC (198.4 vs 243.6, p<0.0001), the neither Follistatin nor Myostatin: Follistatin ratios differ between subsets, and in active versus inactive disease Figure 2 A-D). On follow-up, the serial Myostatin estimation paralleled change in disease activity.

Figure 2. Serum Myostatin levels in IIM as compared with healthy controls (A) and disease controls (C). Levels in juvenile and adult IIM (D) and in various subsets of IIM (D).

Table 1.

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<th>THE ROLE OF X-LINKED INHIBITOR OF APOPTOSIS PROTEIN (XIAP) IN SYSTEMIC SCLEROSIS</th>
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<td>C. Bergmann1, L. Hallenberg1, B. Merlevede1, C. Dees1, C. W. Chen1, O. Dieter1, G. Schett1, J. Distler1.</td>
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Background: Pathologic activation of fibroblasts is a central feature of fibrotic tissue disease in Systemic Sclerosis (SSc). Although individual key signaling pathways of fibroblast activation such as transforming growth factor β (TGFβ) and WNT/β-catenin signaling have been identified, the consequences of the concomitant upregulation of these pathways and their crosstalk are incompletely characterized. Given the high medical need, the identification of mutual activation and amplification loops of profibrotic signals is essential to identify novel candidates for antibiotic therapies. XIAP (X-linked inhibitor of apoptosis protein) is a ubiquitously expressed member of the IAP protein family which are implicated in the regulation of various cellular functions and tissue turnover. XIAP was recently described to be implicated in WNT/β-catenin signaling and TGFβ signaling.

Objectives: The aim of this study is to characterize the role of XIAP in fibrotic disease.

Methods: XIAP-expression was analyzed by qPCR, IF and Western blot. XIAP was targeted pharmacologically and with siRNA. The activation of WNT/β-catenin signaling was assessed by analyses of WNT target genes, by TOPflash/FOPflash luciferase reporter assay and in reporter mice. In vivo, XIAP inhibition was analyzed in two different models of fibrosis.

Results: The expression of XIAP is increased in the skin of SSc patients compared to matched healthy individuals with a prominent expression in fibroblasts. The overexpression of XIAP is more pronounced in SSC patients with diffuse and active skin fibrosis compared to SSC patients with limited and inactive disease. The overexpression of XIAP is also reflected in several experimental fibrosis models: the model of scleroderma-dermatous graft versus host disease, the model of bleomycin induced skin fibrosis and Topoisomerase I induced fibrosis (TopoI) mice. TGFβ induces the expression of XIAP in vitro and in vivo and treatment with the TGFβ1 receptor antagonist SD208 reverses the TGFβ induced expression of XIAP. Inhibition of XIAP with embelin or siRNA reduces the TGFβ induced activation of fibroblasts with reduced collagen release and reduced expression of myofibroblast markers. In addition, XIAP inhibition reverted the activated fibroblast phenotype in SSc fibroblasts with reduced expression of stress fibers and αSMA. The anti-fibrotic effects of XIAP inhibition occurred in non-toxic doses as demonstrated by MTT and by TUNEL staining. In vivo, inhibition of XIAP reduced skin fibrosis in the models of bleomycin induced skin fibrosis and in TopoI-induced skin and lung fibrosis as assessed by analyses of dermal thickening, dermal hydroxyproline content and by analysis of myofibroblast differentiation. Mechanistically, XIAP inhibition reduced the activation of WNT/β-catenin signaling as demonstrated by TOPflash reporter assays and by the analysis of WNT target genes.

Conclusion: XIAP is upregulated in SSc fibroblasts and murine SSc models in a TGFβ-dependent manner and promotes fibroblast activation by fostering canonical WNT signaling. Our data suggest that XIAP mediates an amplification loop between TGFβ and WNT/β-catenin signaling. Inhibition of XIAP may thus be a novel approach to target aberrant WNT/β-catenin signaling in fibrotic diseases.

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