


Figure 1. Drug-protein Interaction Network

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AB0135

ASSOCIATION OF STAT1, STAT4 AND JAK2 IMMUNOEXPRESSION WITH THE GRADE OF MONONUCLEAR CELL INFILTRATION IN MINOR SALIVARY GLAND BIOPSYs IN PATIENTS WITH PRIMARY SJÖGREN’S SYNDROME

Keywords: Sjögren syndrome, Prognostic factors

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Background: Signal transducer and activator of transcription 4 (STAT4) is an important pathway for Interferon-γ induction (type 2 IFN signaling). Whereas type 1 IFN signaling pathway depends on signal transducer and activator of transcription 1 (STAT1) and Janus kinase 2 (JAK2). JAK2 is a non-receptor tyrosine kinase that plays a role in signal transduction via others type II receptors including IFN-α, IFN-β, IFN-γ. The IFN signature in Sjögren’s syndrome and overexpression of IFN-1 and IFN-2 in minor salivary glands biopsy (MSGBs) were described.

Objectives: The aim of the study was to analyze the immunoeexpression of STAT4, STAT1 and JAK2 in biopsies of MSGBs depending on the severity of mononuclear cell infiltration (focus score, FS) in patients with primary Sjögren’s syndrome (sSS).

Methods: The study group consisted of 64 patients with sSS aged 48.25±16.47. 89% of the subjects were women. MSGBs were routinely stained with HE for conventional histopathological examination with determination of FS. Monoclonal antibodies were used to determine the immunoeexpression of the STAT1, STAT4, and JAK2, Quantitative Computer Image Analysis of Immunoeexpression (ScanScope AT2 scanner) has been conducted. The percentage of positive immunostaining for each protein was determined in 20 HPF in each group of patients according to FS. Results were analyzed using TIBCO Statistica v. 13.3 Obtained values failed normal distribution assumption, therefore the non-parametric Kruskal–Wallis test with Dunn’s multiple comparison test for post hoc analysis was used to assess the differences between the groups. p < 0.05 were considered to be statistically significant.

Results: 33% sSS patients were characterized by FS=1; 30%-FS= 3; FS=0 - 9%; 3% and 2% of patients revealed FS=5 and FS=6, respectively. In MSGBs JAK2-, STAT1- and STAT4-immunopositive cells were observed. The various nuclear and cytoplasmic immunoeexpression of JAK2, STAT1 and STAT4 in acinar and ductal cells and also in lesions displaying immune-cell infiltration were observed. However the immunoeexpression of STAT4 in the nucleus was relatively rarely found. The percentage of JAK2-, STAT1- and STAT4-positive cells in FS = 0 was significantly lower in comparison with FS equal or greater than 3. However for STAT4, already in FS=2 there was a stronger signal compared to FS=0. The percentage of JAK2- and STAT1-positive cells in MSGBs in FS = 0 was statistically insignificant in comparison with FS = 1 and 2. There was no statistical signifi- cance in the percentage of STAT4-positive cells in patients with FS = 0 in com- parison with FS = 1 (Figure 1). mRNA level of STAT4 in whole blood in patients with sSS revealed the opposite trend. The increase of FS was correlated with the decreased mRNA level of STAT4.

Conclusion: Notably, in the case of STAT4 immunoeexpression increased with increasing FS. Interestingly, mRNA level of STAT4 determined in the whole blood decreased with increasing FS (data not shown).

The question remains why the immunoeexpression of STAT4 in nucleus was rela- tively rarely found, and what is the impact of the dynamics of STAT4-dependent gene expression on the pathogenesis of pSS. In studied patients STAT4 activity dominates in MSGBs infiltrates which confirms the role of this factor in the patho- genesis of pSS.

REFERENCES:


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AB0136

INTERFERON STIMULATED GENES IN CD4 T CELLS ARE ASSOCIATED WITH PYROPTOSIS PATHWAYS IN PATIENTS WITH LUPUS

Keywords: Systemic lupus erythematosus, -omics

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Background: Single cell RNA sequencing (scRNA-seq) of kidney tissues in patients with lupus has led to landscape of complete cellular composition and state of immune and non-immune cells. These studies suggested type I interferon (IFN) signatures prime inflammatory responses. However, there is a lack of knowledge regarding the characteristics of subsets of IFN stimulating genes (ISG) high expressed cells.

Objectives: Here we investigated immune cell compositions between peripheral blood mononuclear cells (PBMCs), skin and kidney tissues in patients with lupus using scRNA-seq. We characterized a subset of ISG high CD4 T cells, which are commonly expressed across PBMCs, skin and kidneys of lupus but not skin of healthy.

Methods: scRNA-seq dataset of PBMCs, skin and kidney tissues in patients with lupus were collected. We integrated and analyzed immune cell compositions from this scRNA-seq dataset. We focused on subset of ISG high expressed CD4 T cells and characterized top 200 genes of this subset.

Figure 1. The percentage of JAK2-, STAT1- and STAT4-immunopositive cells in the human labial salivary glands according to the focus score (FS 0, FS 1, FS2, FS 3, FS 4, FS 5, FS 6) in the patients with pSS "*" < p < 0.05 vs. F0 group; "*" p < 0.001 vs. F0 group

Figure 1. Interferon stimulated genes in CD4 T cells are associated with pyroptosis pathways in patients with lupus.
Results: There is one subset of ISG high expressed CD4 T cells in the lupus PBMCs, kidneys, and skins but not in the healthy skins. This ISG high expressed CD4 T cells have the greatest number of type I interferon signatures and gas- dermin D gene, which is related to pyroptosis of cells, among top 200 genes. We further investigated pyroptosis pathway genes in the subsets of CD3 T cells. Interestingly, the subset of ISG high expressed CD4 T cells have most increased pyroptosis related upstream regulating genes including IRF1, GBP1, CASP4 and CASP1. Furthermore, this subset highly expressed inflammasome gene such as NLRPC3 not NLRPC4.

Conclusion: There are ISG high expressed CD4 T cells across tissues of lupus. Those cells highly expressed pyroptosis pathway related genes. Further investigation is needed to characterize association between ISG and pyroptosis.

REFERENCE:

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AB0137
FIRST-IN-HUMAN INVESTIGATION OF HYDROGEN VOLTAGE-GATED CHANNEL 1 (HVCN1) EXPRESSION AND PYROPTOSIS LEVELS OF MYELOPEROXIDASE AND OXIDIZED DNA (8-OHODG) IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOUS (SLEOX STUDY) – AN INTERIM ANALYSIS

Keywords: Systemic lupus erythematosus, Cell biology

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Background: Within the still poorly understood pathogenesis of systemic lupus erythematosus (SLE) granulocyte neutrophils have been shown to play a key role. In our research on mouse models, we found that important functions of neutrophils are regulated by the hydrogen voltage-gated channel 1 (HVCN1), whose deficiency causes an SLE-like autoimmune disorder in old mice [1]. Among other effects, HVCN1 regulates the secretion of myeloperoxidase (MPO) and the release of neutrophil extracellular traps (NETs) in mice, thereby controlling inflammation. Based on this knowledge and a previous gene array analysis of CD16+ peripheral blood cells from SLE patients that showed reduced HVCN1 mRNA expression in neutrophils [2], we hypothesize that reduced HVCN1 expression leading to increased MPO and NET release might be a pathomechanism observable in humans that could contribute significantly to the pathogenesis and disease activity in SLE.

Objectives: This first-in-human trial prospectively assesses HVCN1 protein expression of leukocytes and plasma levels of MPO and 8-OHdG in correlation with clinical disease activity in SLE patients and matched healthy controls, in order to gain deeper understanding of SLE pathogenesis.

Methods: In this prospective study (ethical approval obtained under institutional Review Board #112/22), 50 patients with SLE and 50 healthy controls are investigated for HVCN1 expression in peripheral blood leukocytes and plasma levels of myeloperoxidase (MPO) and oxidized DNA (8-OHdG), accompanied by application of the SLEDAI-2K assessment for clinical disease activity and recording of further clinical (demographics, patient and treatment history) and laboratory data (autoimmune, urine and routine serum diagnostics). HVCN1 protein expression is quantified by flow cytometry (BD FACS Symphony A5) via targeted HVCN1 staining (Alomone labs anti-HVCN1 rabbit IgG and Invitrogen anti-rabbit IgG Alexa Fluor 488). Previous processing steps include leukocyte staining (CD45, CD14, CD15 and CD16), cell fixation and permeabilization. Reference quantification of HVCN1 protein expression via Western Blot and further mRNA and DNA measurements are planned. MPO and 8-OHdG levels will be quantified by ELISA and mass spectrometry.

Results: The preliminary analysis of 18 SLE patients revealed a statistically significant positive correlation between SLEDAI-2K scores and HVCN1 protein expression (Pearson correlation coefficient: 0.563; p=0.015). Also, within the typical SLEDAI-2K disease activity categories, there was a clear tendency towards ascending HVCN1 protein quantities expressed with every severity category step-up (see Figure 1). Further analysis is ongoing.

Conclusion: Unlike previous studies, our preliminary SLEOX trial data suggests a positive correlation of HVCN1 protein expression and SLE activity. General HVCN1 upregulation following leukocyte activation in response to disease activity (among other factors) could be one explanation. Validation in a larger study cohort is currently underway.

REFERENCES:
[2] Personal communication with Prof Ken Smith and Dr Paul Lyons, Department of Medicine, University of Cambridge, Cambridge, UK, 2022 (Unpublished).

Figure 1. Graphic display of HVCN1 expression across disease activity groups and healthy individuals.

AB0138
CHARACTERIZATION OF SERUM CYTOKINE PROFILE IN PATIENTS WITH ACTIVE LUPUS NPHRIS

Keywords: Cytokines and chemokines, Systemic lupus erythematosus

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Background: Cytokines (CKs) are known to play a role in the pathogenesis of lupus nephritis (LN), yet their role as biomarkers is still debated.

Objectives: To describe the CKs picture in patients with active LN and to assess possible clinical correlates in LN course.

Methods: A prospective cohort study including patients with active biopsy-proven LN was performed. Serum CKs were assessed at the time of diagnosis and at 3 subsequent time points (3, 6 and 12 months) during follow-up. Clinical and serological data were collected for further analysis. BLyS (B Lymphocyte stimulator) and interleukin (IL)-37 were measured by ELISA assays (Quantikine BAFF, R&D, USA, detection limit DL 0.02 ng/mL; IL37 ELISA kit, Adipogen, Switzerland, DL 0.001 ng/mL), IL-2, IL-10, IL-17A and IL-18 (pg/mL) by Luminex multiplex assay (Millipore, USA, DL 0.6 pg/mL for all), according to the manufacturers’ instructions. Measurements for IL-37 and BLyS were matched with sera of sex- and age-matched healthy subjects (HC). Mann-Whitney test was used for comparisons between two independent groups, Wilcoxon log-rank test for paired comparisons at different time points, Spearman’s correlation coefficient for associations between CK and between CK and clinical features.

Results: Twenty-seven patients with active LN (mean±SD age 41.7±14.74, 78.8% women) were included for initial CK analysis at the time of LN activity. At baseline (T0), BLyS levels were significantly increased in LN compared with HC (median [range]; 1.107 [0.326-4.00] vs. 0.563 [0.265-1.409]; p<0.0001); IL-37 was significantly reduced in LN compared with HC (median [range] 0.016 [0.001-0.251] vs. 0.056 [0.011-1.147]; p = 0.0185). At T0, median (range) levels of IL-2, IL-10, IL-17A and IL-18 were: IL-2 0.640 (0-3.782), IL-10 2.60 (0-106.86), IL-17A 2.10 (0-138.20), IL-18 243.10 (28.20-950.30). The longitudinal association study between CK levels of the entire cohort revealed a trend of inverse correlation between IL-37 and IL-18 (r=0.330, p=0.04) and between IL-2 and IL-17A (r=0.470, p=0.001) (Figure 1). A subset of 17 patients (70.6% F , mean age at renal flare 41.75±14.74, 70.6% women) was followed-up prospectively: 7 (41.18%) showed a positive correlation of HVCN1 protein expression and SLE activity.

Figure 1. Characteristics of serum cytokine profile in patients with active lupus nephritis.