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Figure 1. IL-40 overexpression in pSS and pSS-associated lymphoma **A**, Salivary gland biopsies stained for IL-40 showing a marked increase in IL-40 expression in presence of higher focus score. **B**, Parotid gland specimens of pSS-associated lymphoma showing intense staining for IL-40.

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POS0178

GENE SIGNATURE FINGERPRINTS DIVIDE SLE PATIENTS IN SUBGROUPS WITH COMPARABLE BIOLOGICAL DISEASE PROFILES: A MULTICENTRE LONGITUDINAL STUDY

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Background: Even in the hands of experienced clinicians, clinical phenotyping and predicting treatment responses in Systemic Lupus Erythematosus (SLE) patients remains challenging. Therefore, the identification of biomarkers that can help physicians to divide patients into subgroups based upon aberrantly activated pathways is relevant and might guide future treatment strategies. Extensive blood transcriptional profiling has identified various gene modules that seem promising for stratification of SLE patients into subgroups (1). However, the feasibility to implement these complicated and expensive tests for use in the daily practice of routine clinical laboratories is challenging if not impossible.

Objectives: The aim of this study was to develop gene signatures that stratify patients into groups with comparable disease profiles and are feasible to perform in routine clinical laboratories.

Methods: To identify coordinated expression of a set of genes and reduce data complexity, genes from 4 previously described modules (Interferon M1.2, Interferon M5.12, Neutrophil- and Plasmablast (PB)(1)) were measured using real-time quantitative PCR expression on whole blood RNA samples. Subsequently, a principle component analysis was used to select 2-5 indicator genes, that represent a specific signature. Expression levels of these genes were measured in healthy donors (n=42) and samples from two independent childhood-onset SLE cohorts (n=51 and n=20). Scores higher than the mean + 2 S.D. score of healthy controls were defined as high gene signature scores. Based on their expression levels, gene signatures were divided over 14 clusters. Associated clusters were subsequently grouped into three gene fingerprints termed 1) all-signatures-low,

2) only high IFN (M1.2 and/or M5.12) and 3) high PB and/or Neutrophil. Disease activity was measured by the SELENA-SLEDAI score.

Results: All four gene signatures were higher expressed in patients compared to healthy controls and showed a significant correlation with the SLEDAI. The PB signature showed the highest association with disease activity (r= 0.6512, P<0.0001). In longitudinally collected samples, the PB signature was reduced in patients who were on treatment and showed a significant trend with the SLEDAI. When patients were divided into the described gene fingerprints, the highest SLEDAI scores (median score=8) were observed in the high PB-Neutrophil group. The lowest disease activity (median score =2) was observed in the all-signatures-low group. The same distribution was seen when samples from a second time point were divided based on this stratification method and this was also reproduced in samples from an independent SLE cohort (figure 1).

Conclusion: Various gene signatures are associated with disease activity, which underlines the involvement of different pathophysiological mechanisms in SLE. Combining these signatures into gene fingerprints can help to stratify patients into comparable groups and guide individualized treatment choices for patients in the future.

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Figure 1. SELENA-SLEDAI scores based on gene fingerprint distribution. Blue indicates time point one of cohort 1 (n=51); pink indicates time point two of cohort 1 (n=45); red indicates time point one of the replication cohort (n=20)

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POS0179

EXPLORING THE EFFECTS OF A TWO-SCORE INTERFERON SIGNATURE AND RESPONSES TO INTERFERON STIMULUS

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Background: IFN Scores are increasingly important as biomarkers to stratify RMDs and select patients for interferon targeted therapies. Several studies have previously demonstrated that different sets of interferon stimulated genes (ISGs) have different clinical associations. We validated a 2-score system of ISGs expression consisting of Score A (a set of ISGs included in most interferon signature assays) and Score B (a set of less commonly

measured ISGs). Score B consistently demonstrated stronger clinical associations in studies of diagnosis, prediction of progression to SLE in At-Risk individuals, response to rituximab and imaging-proven synovitis. Previous literature suggested that subsets of ISGs were explained by different interferon subtypes.

Objectives: To understand the determinants of IFN Score A and IFN Score B.

Methods: PBMCs and whole blood Tempus from 4 different healthy donors were stimulated with various cytokines and IFNs at 6- and 24-hour time points. RNA from both sample types were extracted and the expression of 26 interferon stimulated genes were measured using TaqMan and normalised to house-keeping gene PPIA. IFN Scores A and B were calculated as previously described [1]. To compare relative increase in expression with each condition, we calculated delta Ct fold change relative to non-stimulated. To represent greater expression as numerically higher positive values, relative expression was calculated as (fold change⁻¹⁰⁰) +100. Independent T-test calculated the significant differences between each condition compared to IFN α stimulation.

Results: Table 1 shows differences between each condition compared with IFN α at the 6-hour time point. In both sample types, all conditions excluding IFN β stimulation were shown to induce significantly lower expression of both scores compared to IFN α ($p < 0.01$). At 6 and 24 hours, IFN α and IFN β showed a strong induction of IFN score A and B. At 6 hours, IFN γ , κ , and λ induced IFN score A and B but weaker than IFN α and β which was shown in both sample types. We did not observe a difference between the expression of these two scores according to interferon subtype. Other cytokines (TNF, IL-1 and IL-6) weakly induced expression of Score A and B. For IL-10 there was a possible discordant effect with increase in expression of Score B, but decreased expression of Score A, however these changes were small in magnitude.

Table 1.

Condition	IFN Score A		IFN Score B	
	Mean relative expression vs. non-stimulated	P value (vs IFN α)	Mean relative expression vs. non-stimulated	P value (vs IFN α)
PBMC				
IFN α	103.4	-	71.8	-
IFN β	97.1	0.373	64.7	0.352
IFN γ	37.5	<0.001	29.5	0.002
IFN κ	16.6	<0.001	3.1	<0.001
IFN λ	24.8	<0.001	11.1	<0.001
IL-1	9.7	<0.001	4.4	<0.001
IL-6	4.5	<0.001	4.1	<0.001
IL-10	-15.2	<0.001	8.0	<0.001
TNF α	9.6	<0.001	1.2	<0.001
Whole Blood				
IFN α	116.7	-	74.1	-
IFN β	116.6	0.997	73.2	0.894
IFN κ	23.0	0.001	7.9	0.003
IFN λ	23.0	0.001	7.6	<0.001

Conclusion: We found a difference in the induction of ISG expression between subtypes of Type I interferon, as well as other interferons and cytokines. However, the relative expression of IFN Score A and IFN Score B is not easily explained by the subtypes of interferon. We have also previously shown that these scores are both similarly expressed comparing different cell subsets. The explanation for the coordinated expression of these ISGs is therefore unclear and future work will explore the scores with a combination of conditions.

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POS0180

TYPE I INTERFERON DEPENDENT HSA-MIR-145-5P DOWNREGULATION MODULATES MUC1 AND TLR4 OVEREXPRESSION IN SALIVARY GLANDS FROM PRIMARY SJÖGREN'S SYNDROME PATIENTS

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Background: Primary Sjögren's syndrome (pSS) is an autoimmune and inflammatory disease that mainly affects the salivary glands (SG) and is characterized by an overactivation of the type I interferon pathway (IFNs I). IFNs I are known to regulate the levels of microRNAs (miRNAs), non-coding RNAs whose levels are altered in pSS. IFNs I can decrease the levels of miR-145-5p, a miRNA with anti-inflammatory roles that has been reported downregulated in SG of from pSS patients [1]. This miRNA has TLR4 and MUC1 transcripts as predicted targets. MUC1 and TLR4 are two proteins overexpressed in SG of pSS patients that contribute, through various mechanisms, to the inflammatory state and glandular dysfunction [2-3]. Thus, we propose that IFNs I may contribute to a self-perpetuating inflammation loop through a hsa-miR-145-5p dependent MUC1 and TLR4 overexpression in the SG of pSS patients.

Objectives: to evaluate whether mRNA levels of MUC1 and TLR4 are modulated by hsa-miR-145-5p in a IFNs I dependent manner.

Methods: 13 pSS patients and 9 controls SG biopsies were analyzed. hsa-miR-145-5p levels were determined by TaqMan assays and MUC1, TLR4, IFN- α and IFN- β mRNA levels by RT-qPCR. Additionally, in vitro assays using type I IFNs and chemically synthesized hsa-miR-145-5p mimics and inhibitors were performed to study its effect on MUC1 and TLR4 expression. JAK1 and STAT1 mRNA levels were also measured.

Results: By Taqman assays we validated the decreased hsa-miR-145-5p levels ($p=0.0001$) in SG of pSS patients compared to controls. The decreased hsa-miR-145-5p levels correlated inversely with the increased mRNA levels of IFN- β ($p=0.0192$) in SG of pSS-patients. The hsa-miR-145-5p downregulation also correlated inversely with the overexpression of its predicted targets MUC1 ($p=0.010$) and TLR4 ($p=0.0004$). In vitro assays showed that IFN- β induces the overexpression of JAK1 ($p<0.0001$) and STAT1 ($p<0.0001$) leading to the downregulation of hsa-miR-145-5p ($p<0.0001$) and increased MUC1 ($p<0.0001$) and TLR4 mRNA levels ($p<0.0001$). Functional assays suggest a regulation of hsa-miR-145-5p on MUC1 and TLR4 expression as MUC1 and TLR4 mRNA levels were decreased in HSG cells transfected with hsa-miR-513c-3p mimic and increased in HSG cells transfected with the miRNA inhibitor.

Conclusion: Our findings suggest that IFNs I could induce the downregulation of hsa-miR-145-5p leading to the overexpression of MUC1 and TLR4 in SG from pSS patients. TLR4 is activated by ectopic mucins in the SG extracellular matrix from pSS patients which induces pro-inflammatory cytokines secretion [3]. Furthermore, the high levels of the MUC1-SEC and MUC1-Y isoforms observed in SG from pSS patients may favor cytokine synthesis through the immuno-enhancing peptide of MUC1-SEC or through the formation of a MUC1-SEC/MUC1-Y complex [4]. Therefore, IFNs I may contribute to the development of SS through amplification and perpetuation of inflammation due to a hsa-miR-145-5p dependent MUC1 and TLR4 overexpression.

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