A MOLECULAR SIGNATURE OF SYSTEMIC LUPUS ERYTHEMATOSUS IN THE CONTEXT OF OTHER AUTOIMMUNE DISEASES

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a very heterogeneous manifestation affecting mainly the skin, kidneys, joints and the central nervous system. Despite advances in research on SLE pathology, its diagnosis remains challenging, especially due to its heterogeneity. Therefore, the demand for biomarkers that contribute to early diagnosis and better disease monitoring is high. Gene expression-based biomarkers represent a powerful tool in several clinical areas and are currently also under intense investigation for SLE.

In this study, we demonstrate the use of GENEVESTIGATOR® for generating an SLE gene expression signature from blood samples of SLE patients and healthy donors. Furthermore, this signature is co-analyzed with a compendium of gene expression data sets from a large variety of different autoimmune diseases. This is an important approach to better understand molecular changes associated with SLE and to differentiate disease-specific from common autoimmune-related changes.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a broad clinical phenotype mostly affecting the skin, kidneys, joints and the central nervous system. The disease etiology as well as its progression are very heterogenous and include environmental and genetic factors that lead to the loss of tolerance, chronic autoantibody production, and immune complex deposition (Tsokos et al., 2016). Bearing in mind the heterogeneity of SLE, it is not surprising that diagnosing and monitoring the disease is complex. As there is a strong need for molecular biomarkers that would allow a faster and more precise diagnosis of SLE, several studies characterizing blood transcriptional profiles of SLE patients have been recently performed (Nagafuchi et al., 2019). Evidence indicates that the blood transcriptional profile reflects well the immune status of patients with autoimmune diseases, especially for systemic ones, such as SLE (Berry et al., 2010; Banchereau et al., 2016; Ding et al., 2018). Moreover, the ease and low invasiveness of blood collection in the clinic make it a convenient

tissue for diagnostics. In most of the studies mentioned above, transcriptome analyses were performed on a single study level, which has the limitation that results cannot easily be compared to other related studies.

In this study, we describe how results obtained from an individual SLE study are co-analyzed with a broad compendium of high-quality publicly available transcriptomic data from different autoimmune diseases using GENEVESTIGATOR® (Hruz et al., 2008). Such a data-driven approach offers the possibilities of discovering valuable insights into gene expression changes associated with SLE and investigating in an unbiased way if these changes are shared with other autoimmune diseases.

RESULTS AND DISCUSSION

The GENEVESTIGATOR® compendium contains numerous deeply curated, high-quality microarray and RNA-Seq studies investigating gene expression changes in blood samples of SLE patients.

One RNA-Seq study (HS-02571, GSE112087) was selected to compute an SLE gene expression signature. This signature consists of 155 up- and 195 down-regulated genes and their corresponding fold-change values. Genes and values were obtained from a differential expression analysis of blood samples from adult SLE patients with no experimental treatment compared with healthy donors (Figure 1A).

The genes of the obtained signature were further analyzed with the Gene Set Enrichment tool of GENEVESTIGATOR®. This tool allows to compare a list of genes with other gene sets, e.g. gene sets from Gene Ontology annotations. The top-30 most up-regulated genes from the SLE gene expression signature were found to significantly (p-value <0.001) overlap with gene sets of type I interferon response and with genes associated with an anti-viral response (Figure 1B). These genes include, among others, IFI27 and IFI6. This is in line with the SLE literature, which shows that these responses are involved in the pathogenesis and manifestation of SLE (Crow and Ronnblom, 2019).

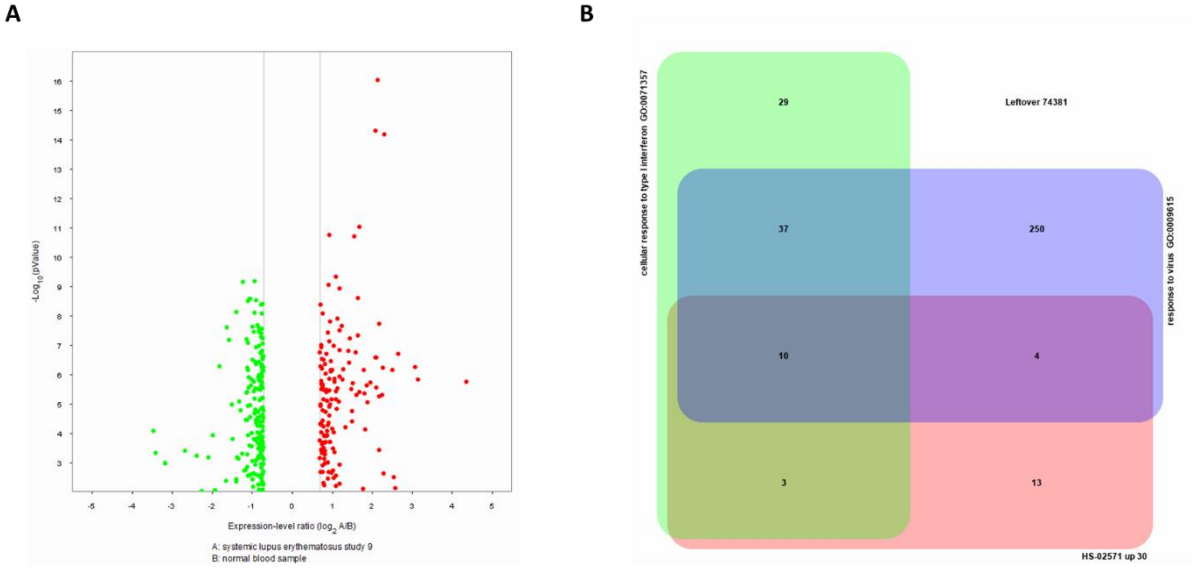

Figure 1. Single-experiment analysis of blood samples from SLE patients compared to healthy controls. **A**: Volcano plot showing differentially expressed genes (up-regulated in red, down-regulated in green) that were used as SLE gene expression signature. **B**: Gene Set Enrichment analysis with Gene Ontology (GO) gene sets show that a large proportion of the 30 top up-regulated genes in SLE (red) are type I interferon response genes (green) and viral response genes (blue).

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In a next step, the SLE blood signature was investigated in the context of other autoimmune diseases. In the Hierarchical Clustering tool of GENEVESTIGATOR®, all genes and conditions of a data selection were grouped based on the similarity of their expression profiles. As GENEVESTIGATOR® allows to compare gene sets and signatures across all supported platforms, the identified SLE genes were analyzed on the data compendium of blood samples with the widest range of different autoimmune studies (HS_AFFY_U133PLUS_2 platform).

Figure 2 shows the results of the hierarchical clustering for comparisons between untreated blood samples from diseased patients versus healthy controls (disease/healthy) across the top and bottom 30 genes of the SLE blood signature supported on this microarray platform. The type I interferon response genes of the SLE blood signature are highlighted in blue letters in the dendrogram. The blood gene expression profile of SLE patients closely resembles the profiles of other autoimmune diseases, such as the profile of scleroderma and rheumatoid arthritis (RA) patients (the lower three yellow boxes). On the other hand, the profile of another autoimmune disease, systemic-onset juvenile idiopathic arthritis (SoJIA) seems to be different from SLE, scleroderma and RA for the genes investigated. This is in agreement with the existing literature that describes an interferon response in blood of SLE, scleroderma and RA patients, but not SoJIA patients (Wu and Assassi, 2013; Gilbert and Punaro, 2014; Rodriguez-Carrio et al., 2017).

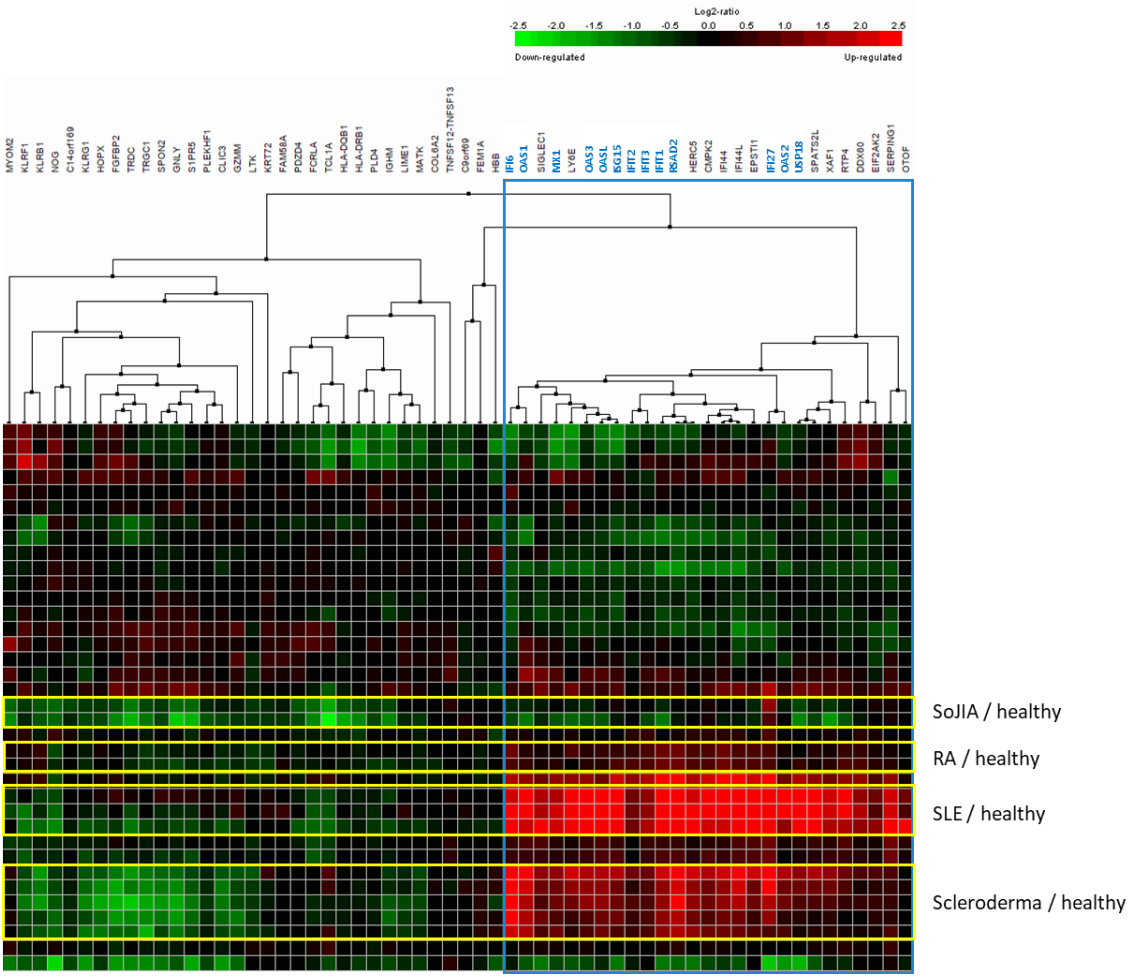

Figure 2. A compendium-wide search for similarities between a blood transcriptomic profile of SLE and other autoimmune diseases. The Hierarchical Clustering tool of GENEVESTIGATOR® was used to group the top and bottom 30 supported genes of the SLE signature (columns) and blood samples from untreated diseased versus healthy comparisons of autoimmune diseases (rows). The gene cluster containing the interferon response-associated genes is framed in blue, while some comparisons are framed in yellow.

Next, we wanted to investigate the change of gene expression of the SLE blood signature genes upon common treatments for autoimmune diseases. Figure 3 shows the results of the hierarchical clustering for comparisons

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between blood samples from diseased patients that underwent treatment versus controls (treated/control). Again, type I interferon response genes of the SLE blood signature are highlighted in blue letters in the dendrogram.

We observed a big cluster of comparisons with an opposite blood gene expression profile compared to the SLE/healthy, scleroderma/healthy and RA/healthy profiles (yellow boxes). Typically, these comparisons were from patients suffering from autoimmunity that were treated with a medication that blocks the interferon response, such as type I interferon-blocking monoclonal antibody (anti-IFNAR) or interferon alpha kinoid (IFN-K).

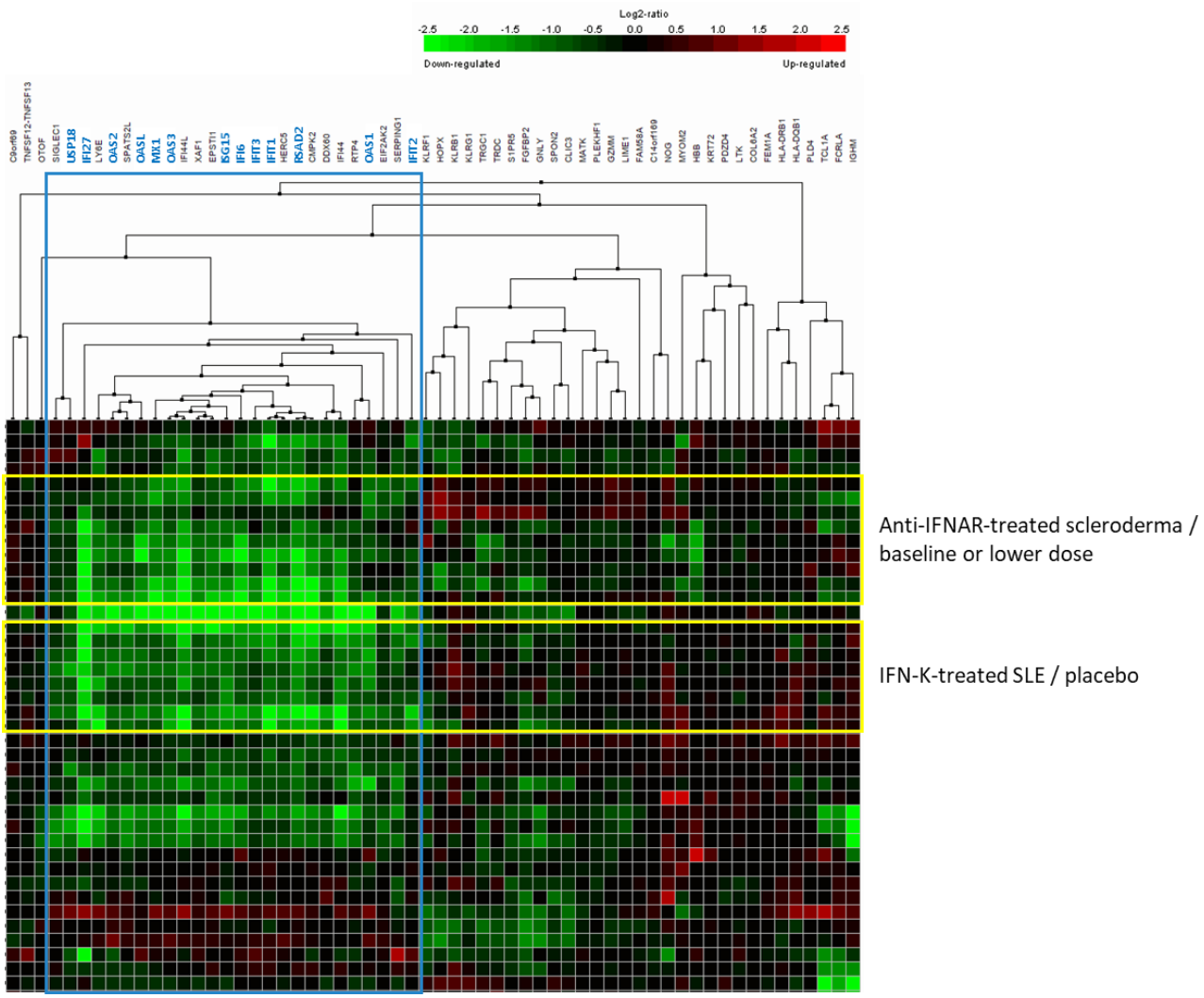

Figure 3. A compendium-wide search for complimentary blood transcriptomic profiles of SLE and other autoimmune diseases. The Hierarchical Clustering tool of GENEVESTIGATOR® was used to group the top and bottom 30 supported genes of the SLE signature (columns) and blood samples from treated diseased versus control comparisons of autoimmune diseases (rows). The gene cluster containing the interferon response-associated genes is framed in blue, while some comparisons are highlighted in yellow.

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At last, we sought to assess the overall blood transcriptional profile of SLE patients as compared to patients suffering from SoJIA on a single-study level, since they showed a distinct regulation of type I interferon response genes in a compendium wide comparison (Figure 2). For this purpose, a study was chosen (HS-02946, GSE19491) that comprised blood samples from patients suffering from different diseases, including SLE and SoJIA. These samples were analyzed using the Dimension Reduction tool of GENEVESTIGATOR® (Figure 4). In this tool, the multi-dimensional gene expression data of an experiment is visualized in a two-dimensional plot enabling the identification of clusters of samples with similar transcriptional profiles.

Several clusters were identified (Figure 4). Blood samples from SLE patients clustered separately from those of SoJIA patients and those from patients suffering from bacterial infections (pulmonary tuberculosis, Staphylococcus and Streptococcus infections). This is in accordance with our previous observations (Figure 2), where type I interferon response genes were shown to be regulated differently in these two diseases. Interestingly, we were able to distinguish two separate SLE clusters representing adult and pediatric patients. When taking a closer look, especially at the pediatric SLE cluster, a significant heterogeneity was observed, which is in agreement with the heterogenous nature of the disease (Tsokos et al., 2016).

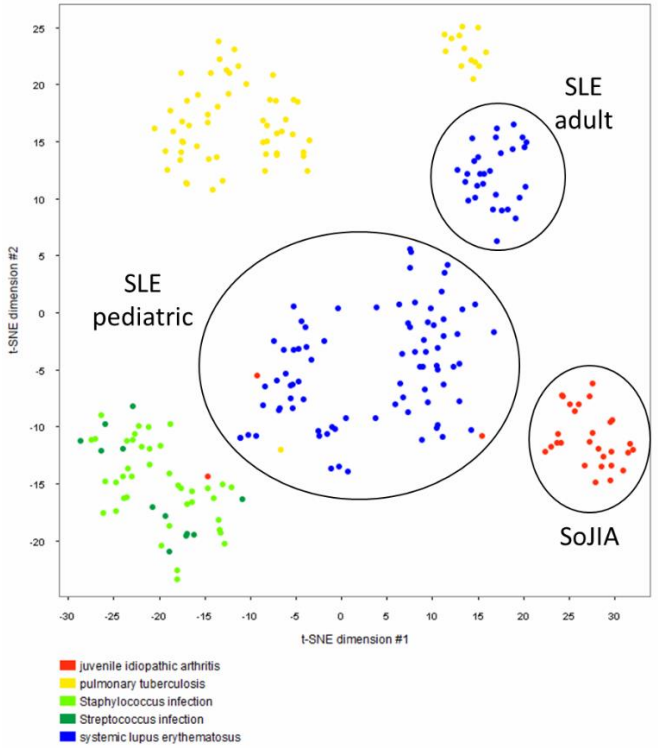

Figure 4. Clustering of patients suffering from SLE and other diseases using the Dimension Reduction tool of GENEVESTIGATOR (based on the t-SNE algorithm).

CONCLUSION

High-throughput microarray and RNA-Seq technologies facilitate gene expression analyses of a broad spectrum of conditions, including diseases, effects of environmental factors, or drug treatments. GENEVESTIGATOR® is an effective analytical tool that contains high-quality curated gene expression data and allows compendium-wide analyses for disease mechanism investigations and drug-target discovery. In this example study, we demonstrated how GENEVESTIGATOR® was utilized to generate a blood gene expression signature for SLE by

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comparing untreated patients to healthy controls. This signature was then further investigated in the context of other autoimmune conditions across different data compendia. We showed that some transcriptional changes (type I interferon response genes) induced by SLE are similar to those found in other autoimmune diseases (e.g. scleroderma) and different from other autoimmune conditions (e.g. SoJIA). We also showed that these changes are reversed by treatments targeting those pathways (interferon signaling blocking agents).

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SELECTED DATA AND SETTINGS FOR GENEVESTIGATOR®

Differential Expression tool: Data selection: HS-02571
Settings: FDR 0.05; |Log ratio|>0.7
Gene Set Enrichment tool: Gene set selection: HS-02571 (30 most up-regulated genes), Gene Ontology annotations gene sets
Hierarchical Clustering tool (Perturbations): Data selection: HS_AFFY_U133PLUS_2 (autoimmune diseases filtered by Research area, blood samples filtered by Anatomy, filtered by treatment status)
Genes: HS-02571 (37 top and 60 bottom genes, this results in 30 genes each on the AFFY_U133PLUS_2 platform)
Settings: Pearson Correlation
Dimension Reduction tool: Data selection: HS-02946 (diseased blood samples) Settings: Perplexity 10

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CONTACT INFORMATION

For any questions or to learn more about GENEVESTIGATOR®, please write to info@nebion.com or visit genevestigator.com.

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