Identification of Subgroups of Systemic Lupus Erythematosus Patients based on Autoantibody Profiling

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Introduction

Systemic lupus erythematosus (SLE) is characterized by extensive immune system aberrations leading to the production of autoantibodies of which many are directed towards nucleic acids (anti-dsDNA) and nuclear protein antigens (ANA). These antibodies potentially contribute to pathological processes affecting skin, kidney, neurological system or heart and lung. The clinical and serological diversity of SLE presents important challenges in the diagnosis of the disease as well as affects the clinical development of new SLE therapeutics.

Luminex bead-based antigen arrays were employed for in-depth characterization of the autoantibody reactivity of SLE as a source to develop improved diagnostic and patient stratification tests for SLE.

Material and Methods

The SeroTag® technology utilizes the bead-based Luminex xMAP technology which enables to measure the reactivity of autoantibodies (AABs) to thousands of different antigens in a single step measurement (Fig. 1). The flexible design accelerates rapid and efficient validation of new biomarker candidates.

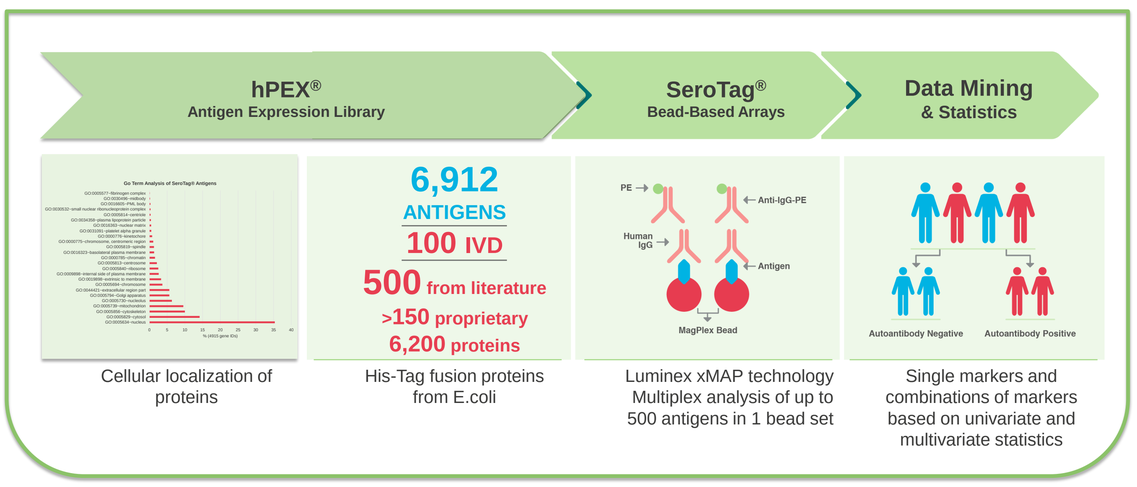

Fig. 1: SeroTag® process

Study Design

A discovery phase in which 130 SLE samples were profiled against rheumatoid arthritis (RA) and healthy controls (HC) was followed by a verification phase in which 100 SLE samples were re-analyzed and tested against HC and a set of samples from other autoimmune diseases (AID) including systemic sclerosis (SSc), ankylosing spondylitis (SPA) and RA. The results from the discovery and verification phase were combined and 296 unique antigens identified. After applying a double filtering procedure with fold change and p-value, 74 putative diagnostic antigens were identified. Further analysis was carried out to identify putative autoantigens for L. nephritis. In total, 85 L. nephritis antigens were identified of which 23 antigens were found in both studies.

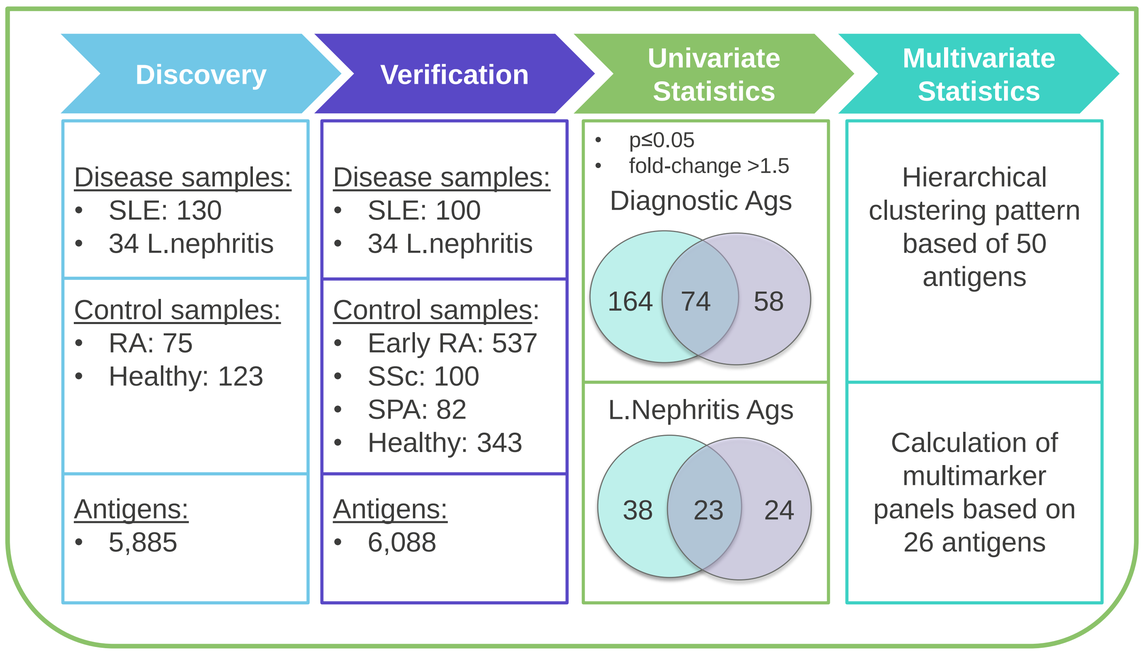

Fig. 2: Strategy for discovery and verification of novel SLE autoantigens

Results

Hierarchical cluster analysis was performed to define natural groupings of antigens and patients. The relative strength of reactivity of each antigen in individual patient samples is shown in a heat map by color intensity (yellow) above the cutoff values (black) (Fig. 3). AAB reactivity to 26 confirmed antigens emerged as six clusters. The percentage of AAB-positive SLE patients relative to HCs is shown in Fig. 4.

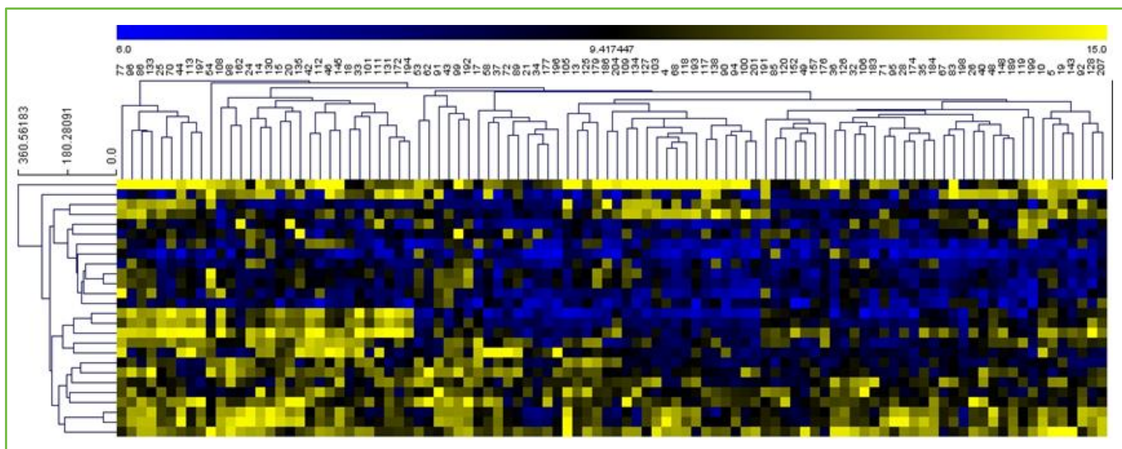

Fig. 3: Natural grouping of patients and antigens

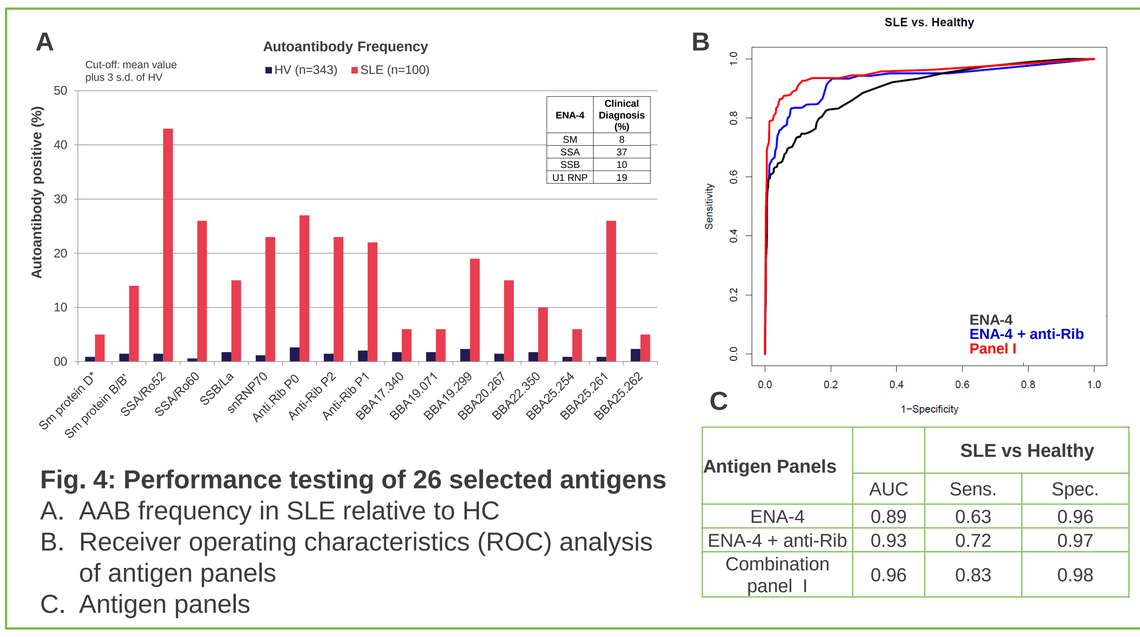

Fig. 4: Performance testing of 26 selected antigens
A. AAB frequency in SLE relative to HC
B. Receiver operating characteristics (ROC) analysis of antigen panels
C. Antigen panels

The 26 selected antigens including a established and new antigens were combined to marker panels. Sequential addition and different combinations of antigens to a panel of known SLE antigens resulted in a stepwise improvement of the classification performance (Fig. 4).

Secondly, putative L. nephritis antigens were tested in combination with established and new diagnostic markers. Hierarchical cluster analysis yielded novel antigen clusters of which one cluster was enriched in L. nephritis antigens (Fig.5).

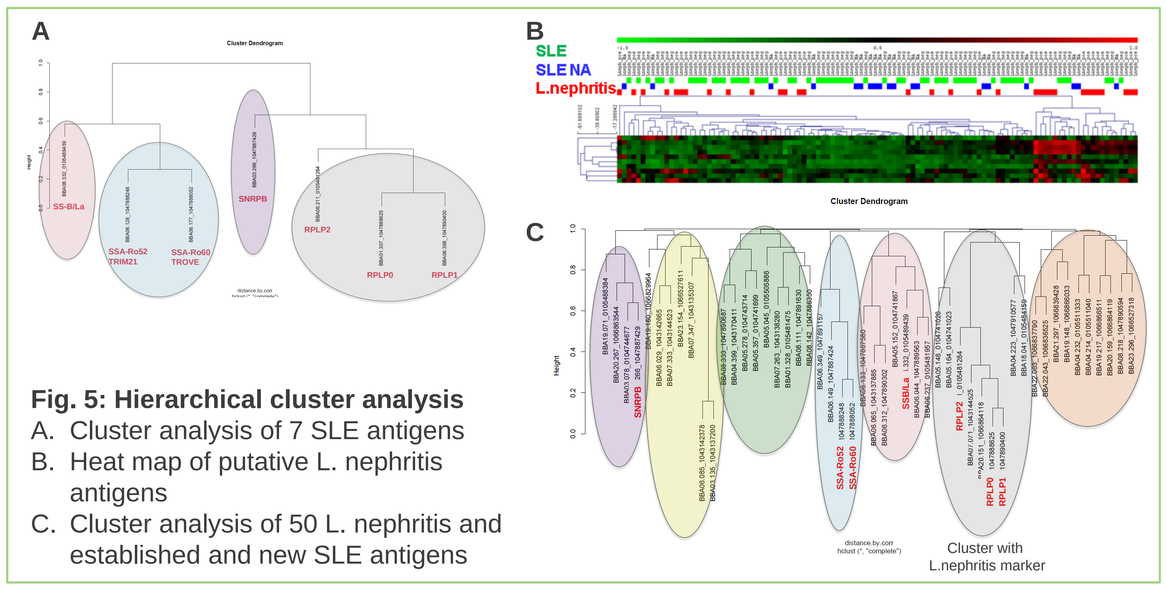

Fig. 5: Hierarchical cluster analysis
A. Cluster analysis of 7 SLE antigens
B. Heat map of putative L. nephritis antigens
C. Cluster analysis of 50 L. nephritis and established and new SLE antigens

Conclusions

Comprehensive profiling of SLE sera enabled the in-depth characterization of the autoantigen repertoire of SLE patients. The combination of established and new antigens significantly increased the sensitivity to diagnose SLE. Based on their autoreactivity profile SLE sub-groups were revealed, one cluster included autoantibodies significantly associated with L. nephritis. However, further studies are needed to link the remaining clusters to clinical or drug response profiles.

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