High-Throughput Screening Discovers Novel Autoantibodies in Autoimmune Diseases: The SeroTag Approach in Systemic Lupus, Systemic Sclerosis and Rheumatoid Arthritis

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EULAR2016 THU0242

Introduction

- Diagnostic biomarkers are decision-making tools in clinical lab routine and are of growing importance for clinical management of patients.
- In autoimmune diseases, one class of biomarkers are autoantibodies (AAB) directed against human autoantigens. Apart from diagnostic antigens used in clinical routine, additional AAB reactivities to more than 100 human antigens are described in literature. Obviously, the autoimmune profile of humans covers a huge number of AABs, which display an enormous resource to identify novel marker candidates.

Objectives

- Here we describe a new screening platform SeroTag for screening of novel AABs in autoimmune diseases.
- By offering thousands of human antigens to serum samples of several autoimmune diseases a comprehensive landscape shall be drawn to identify diseases and disease subgroups according to their intrinsic, highly differentiated autoantibody pattern.

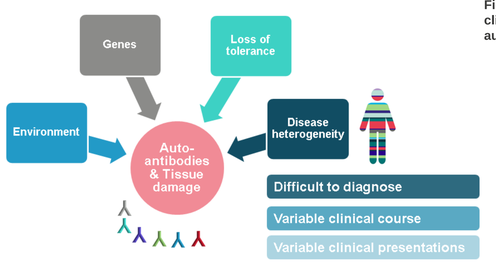

Fig. 1: Serological and clinical heterogeneity of autoimmune diseases

Methods

SeroTag utilizes over 7,000 human proteins as antigen collection in bead-based suspension arrays (Luminex FlexMap 3D) to allow for high throughput serum sample processing with high accuracy, followed by advanced data mining procedures. We screened over 4,000 serum samples from patients with autoimmune diseases such as SLE (n= >500), SSc (n= >250), RA (n= >500), and healthy individuals (n= >350) to confirm known and to discover novel autoantibodies. Recombinant antigens were covalently coupled to magnetic, color-coded beads and serum samples were incubated with 20 multiplex bead mixes each representing hundreds of antigens. Univariate and multivariate statistical analyses were performed to reveal significant antigens and to define correlation of antigens with clinical parameters and amongst themselves.

Results

Identification of Novel Autoantibodies

In SLE, SSc and RA novel autoantigens were discovered in independent discovery and validation studies. Antigens showing reproducible, significant reactivity compared to active and passive controls were selected in a stepwise marker refinement approach.

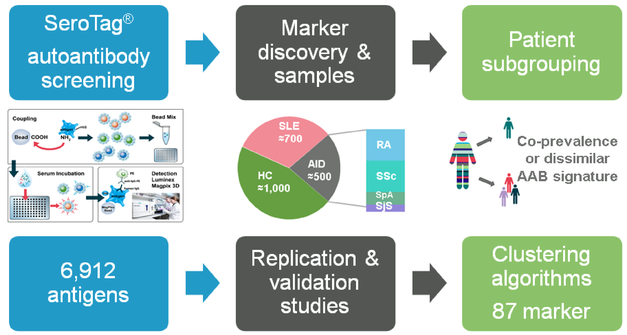

Fig. 2: Discovery and validation of autoantibody markers in autoimmune diseases

Autoantibody Reactivity Signatures

Based on individual marker patterns, patients either belong to clusters defined by characteristic markers, or are phenotypically more overlapping with each other. Subgrouping patients by signatures will allow for optimized clinical management.

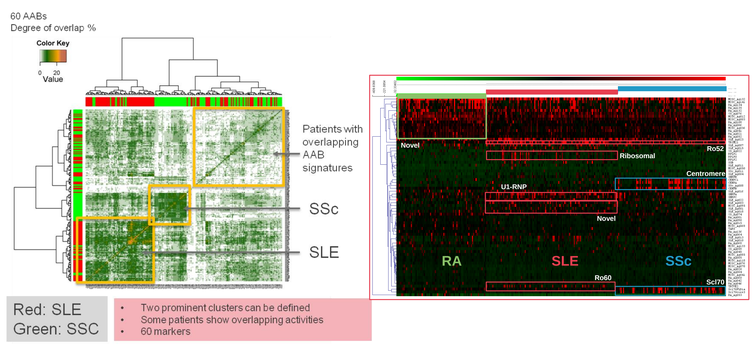

Fig. 3: Autoantibody signature reactivity
A) Cluster analysis of SSc and SLE patients using 60 AABs (Ward, Manhattan distance, markers dichotomized)
B) Cluster analysis of multiple AIDs illustrating AAB reactivity (Manhattan, log2 transformed MFI values, centered)

Kohonen Maps

Self-organizing Maps (SOM) demonstrate the utility of autoantibody panels to outline an autoimmune landscape, differentiating not only different AIDs but also for subgroup formation within diseases.

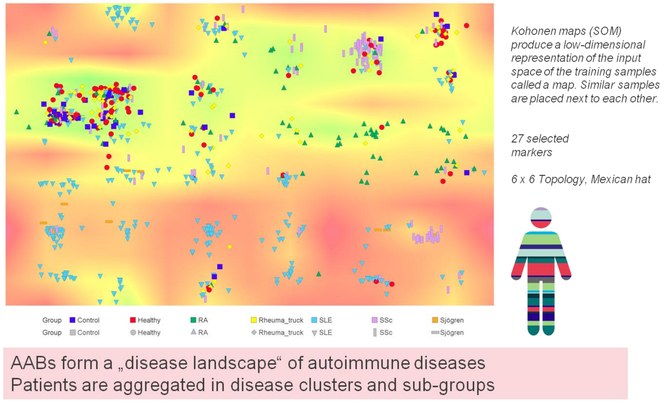

Fig. 4: Kohonen map analysis of AID patients based on 27 selected AAB reactivities
The different serum donor groups are healthy controls (blue, red, yellow), SLE (light blue), SSc (pink), SjS (orange).

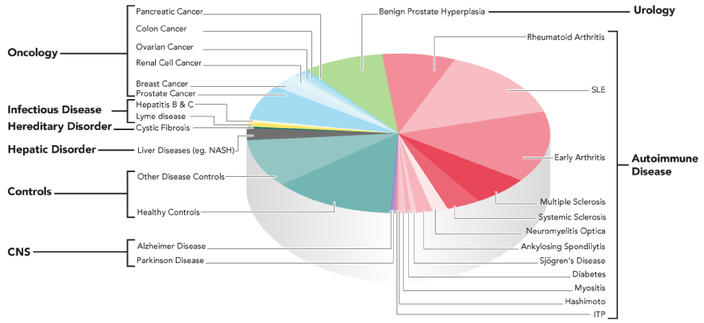

Fig. 5: Disease Areas
A total of more than 14,000 patient samples from numerous therapeutic areas and indications were tested to date.

Conclusions

Discovery approaches for autoantigens in autoimmune diseases show great promise to further detail the autoimmune landscape. SeroTag screening is a valuable tool for “omics”-type biomarker discovery and verification. Novel autoantigens were discovered and validated in RA, SLE, SSc which show potential for improved and earlier diagnosis, differential diagnosis, and disease subgrouping.