Discovery and Validation of New Autoantibody Biomarker in Systemic Lupus Erythematosus

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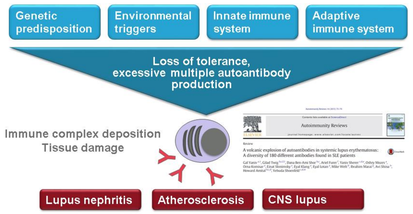
Introduction

- The extreme heterogeneity amongst SLE patients is a major obstacle for predicting disease manifestations and for developing effective therapies.
- In order for this to happen, there is a clear need for diagnostic biomarkers that enable precise disease characterisation, patient stratification and response prediction.
- Characterization of the autoantibody repertoire in SLE might yield novel biomarkers supporting a personalized disease management approach.



Diversity of autoantibodies may be exploited to develop novel SLE diagnostics

Fig. 1: Autoantibody-mediated pathogenesis of SLE

Methods

To identify relevant autoantigens in autoimmune diseases, we developed a bead-based array platform using the Luminex® xMAP® technology. In the current version, SeroTag® enables to detect antibodies against 6,912 antigens (well-described and novel) in parallel with small sample volume requirements (25 µl per sample and screen).

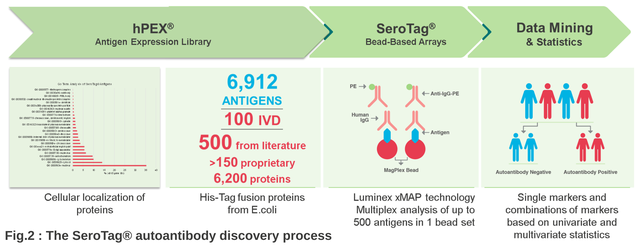


Fig.2 : The SeroTag® autoantibody discovery process

In SLE more than 100 autoantibodies have been reported, which are characterized by overlapping specificities with other rheumatic diseases. Given the inherent heterogeneity of SLE, the multiplexing approach represents a powerful tool to compare autoantibody specificities in SLE and control samples. During discovery serum samples from 130 SLE patients, 794 rheumatic disease controls (systemic sclerosis/SSc, rheumatoid arthritis/RA, early RA, ankylosing spondylitis) and 343 healthy controls (HC) were analyzed.

Results

We previously reported the parallel profiling of autoantibodies targeting well-described and novel antigens in four autoimmune diseases using a bead-based Luminex platform [1]. IgG autoantibody reactivity was observed against 166 antigens targeting well-described and novel antigens. The selected antigens were re-arrayed to construct targeted arrays for marker validation in a new SLE cohort (SLE II). Consistent autoantibody reactivity against 46 antigens was found (p-value <0.05 and Cohen's d effect size >0.3). Fig.3 shows the study design and a graph of the p-values and frequency of autoantibodies in SLE II samples. Marker with an observed frequency >10 % in SLE patients were selected for further investigations.

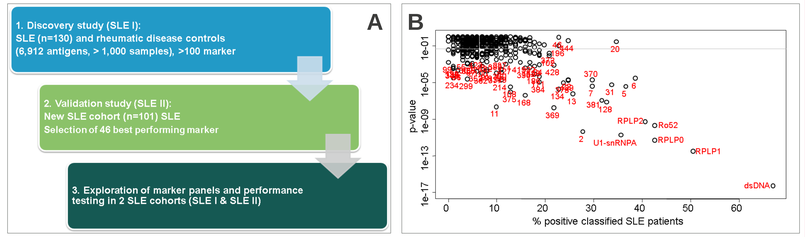


Fig. 3: Discovery and validation of SLE-associated autoantibodies
A) Study design; B) Plot of the autantibody frequency (95% quantile cut-off value of the control group) versus p-value calculated for each antigen in the validation cohort

Fig. 4 shows a heat map of the autoantibody profiles in SLE samples in comparison to healthy and rheumatic disease controls. Well-described and novel antigens are shown. Broad and overlapping autoantibody reactivity was found against SSA/Ro52 (TRIM21 antigen), whereas anti-ribosomal P antibodies were highly specific for SLE. Interestingly, new autoantibodies were found that target proteins involved in apoptotic processes, which might be exposed on the surface of apoptotic cells in SLE patients.

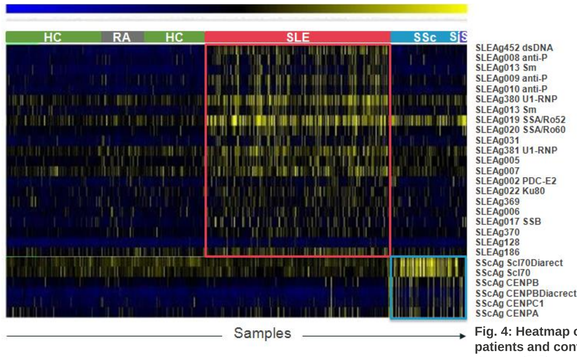


Fig. 4: Heatmap of autoantibody profiles in SLE patients and controls (HC, RA, SSc, Sjögren's disease/SjS)

To gain more detailed information on target antigens, we employed the Gene Ontology (GO) Database and STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) to retrieve and visualize protein-protein interaction, molecular function and pathway representation. Fig. 5 shows all antigens, which are represented by nodes in the resulting graph.

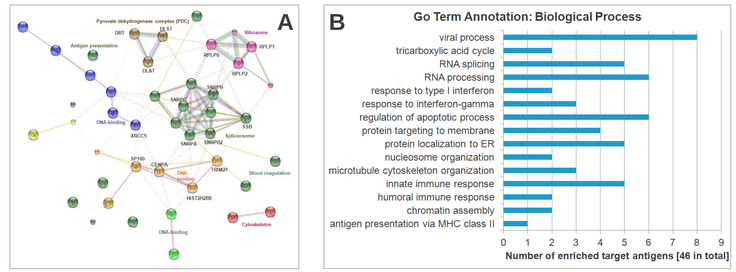
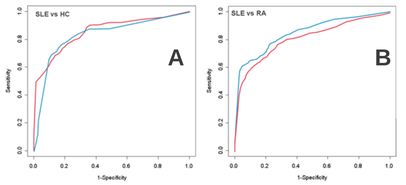


Fig. 5: A) Protein-protein interaction analysis; B) Analysis of biological pathways

Fig. 4 shows that SLE patients have multiple autoantibodies, which are only detected in subsets of SLE patients. We explored the utility of new markers to identify SLE by constructing different combinations of multi-marker panels. Fig. 6 shows an example, where the classification performance of an 11-marker panel was explored in the discovery cohort (SLE I) and in the validation cohort (SLE II). The marker panel combines new and existing autoantibodies (Sm, SSB, SS-A, U1-RNP). Receiver Operating Characteristic (ROC) analysis was carried out to find the optimum sensitivity, specificity and Area Under the Curve (AUC). The selected marker panel shows most promising performance. However, additional antigen optimization and validation steps of individual markers are needed until a final marker panel can be constructed.



	AUC Mean (CI)		Sensitivity Mean (CI)		Specificity Mean (CI)	
SLE vs HC						
SLE I	0.87	(0.86-0.89)	0.79	(0.77-0.82)	0.83	(0.81-0.85)
SLE II	0.87	(0.86-0.88)	0.76	(0.74-0.79)	0.82	(0.80-0.84)
SLE vs RA						
SLE I	0.89	(0.88-0.89)	0.78	(0.76-0.80)	0.81	(0.80-0.83)
SLE II	0.84	(0.83-0.85)	0.72	(0.70-0.74)	0.80	(0.78-0.82)

Fig. 6: Classification performance of an 11-marker panel
A) SLE vs HC and B) SLE vs RA

Conclusion

Using a multiplex platform we have discovered novel SLE-associated antigens. The reproducibility of the autoantibody reactivity was verified and validated in new SLE samples applying a stepwise marker refinement approach. This approach yielded novel markers combinations, which may improve the diagnostic sensitivity and specificity.

References: (1) Lueking A. et al. Ann Rheum Dis 2013;72:A535