Identification of Novel Scleroderma–Associated Antigens and Development of an Autoantibody Assay Panel Enabling Their Subsequent Validation

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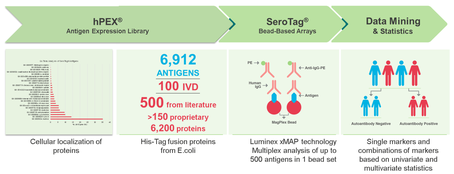
Background:

Systemic sclerosis (SSc) is a systemic autoimmune disease that clinically manifests as progressive fibrosis of the skin and internal organs. SSc is associated with the presence of several autoantibodies, of which the three most important SSc-specific autoantibodies, anti-centromere antibodies (ACAs), anti-topoisomerase antibodies (ATAs), and anti-RNA polymerase III antibodies (ARAs) are found in over 50% of SSc patients and are generally exclusive of the other. Autoantibody specificities are strongly associated with pattern of organ involvement and disease outcome, making autoantibodies an essential tool in the clinical management of SSc. This highlights the attractivity for additional specific and sensitive diagnostic, prognostic and therapeutic response biomarkers in SSc. We have recently conducted high-content autoantibody profiling studies of SSc, systemic autoimmune diseases (AID), and healthy controls and found in addition to diagnostic autoantibodies novel SSc-associated autoantibodies



The SeroTag® autoantibody discovery process is based on a bead-based array screening of recombinant human autoantigens

Objectives:

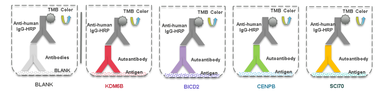
In this study, novel SSc-associated autoantibody marker candidates were re-evaluated using complementary autoantibody measurement platforms and assay formats. Autoantibody specificities were analyzed in independent samples and their association with clinical subsets investigated.

Methods

A systematic and undirected approach was undertaken to screen for autoantibodies in human serum samples of 100 individuals with SSc and related overlap syndromes using 7,000 recombinant protein targets. Active and passive control groups comprised healthy controls and serum samples of other systemic autoimmune diseases including systemic lupus erythematosus (SLE), and early rheumatoid arthritis (RA). Seven antigens were selected based on univariate statistics, frequency in SSc and control groups and signal strength (high reactivity). Afterwards, panel tests were developed for candidate antigens and the performance analyzed in comparison with the Luminex multiplex system in additional SSc and AID samples. The discovery cohort comprised n=100 SSc cases of which 86 met the ACR/EULAR SSc classification criteria and were subclassified as diffuse SSc (n=32), limited SSc (n=50), scleroderma overlap syndrome (n=9), undifferentiated SSc (n=3) and special form (n=1) (Le Roy et al. 1988; Hunzelmann et al. 2008). The verification cohort comprised of n= 80 SSc samples and different SARD control samples including AIM (n=53), SLE (n=67), SjS (n=26), RA (n=77) and UCTD (n=16).

	SLE	SSc				Early RA (<6 Month)	SPA	Healthy
		Limited	Diffuse	Overlap Syndrome	No data on subform			
Number	100	50	32	9	9	537	82	343
Age (Year)	39.8 +/-11.9	61.53 +/- 16.97	53.88 +/- 14.75	51.78 +/- 9.18	47.56 +/- 16.97	56.8 +/- 14.3	43.7 +/- 10.1	47.7 +/- 11.7
Female	83	50	32	8	9	62.2	15.9	58.3
ANA positive	100	47	32	9	7	N.D.	N.D.	N.D.
Anti-CENP	N.D.	30	4	2	2	N.D.	N.D.	N.D.
Anti-Scl70	N.D.	9	20	1	2	N.D.	N.D.	N.D.

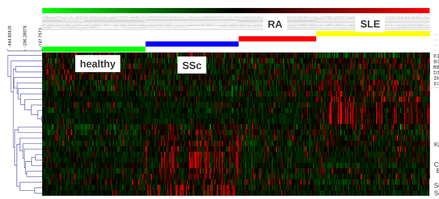
Left: Demographic data of discovery data set. The samples have been selected to represent different cohorts of SSc patients (e.g. anti-Centromere+, Scl70+)



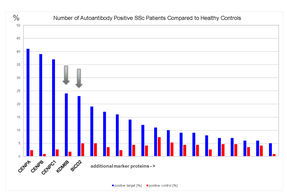
Right: ELISA test principle for the developed multi ELISA prototype.

Results

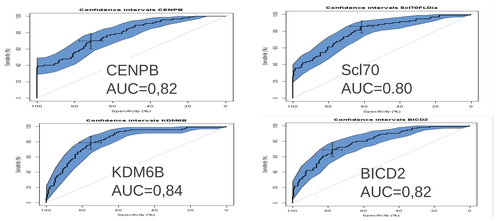
Sera of 100 SSc patients with limited or diffuse SSc or overlap syndromes were tested for established and novel autoantigens. 30% of SSc patients were previously tested negative for ACA and anti-Scl70. The frequency of established and novel autoantibodies in the test cohort ranged from 40% to 10% in descending order: 1. CENPB: 40%, 2. Scl70: 36%, 3. TRIM21 (Ro52): 27%, 4. KDM6B: 28%, 5. BICD2: 27%, 6. PASSC1: 17%, 7. PASSC2: 15%, 8. PASSC3: 13%, 9. PASSC4: 11% and 10. PASSC5: 4%. While autoantibodies to antigens 4-7 were more frequently observed in limited SSc, autoantibodies to antigens 8-10 were more abundant in diffuse SSc.



Cluster analysis of a set of patient samples (green: healthy, blue: SSc, red RA, yellow: SLE) of different candidate antigens after technical replication of the measurement in a targeted analysis of selected antigens. The novel antigen candidates „KDM6B" and „BICD2" exhibit reactivity profiles, which cluster together with the anti-centromere protein reactivities

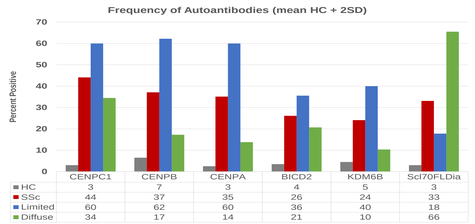


*Frequency of the presence of autoantigens as observed during the serotag discovery screen for the 100 SSc samples. The novel biomarker targets referred to as KDM6B and BICD2 protein fragments are the most prominent marker candidates in the investigated SSc population compared to healthy controls exhibiting app. 20 % positive samples (level > 2*SD of control samples)*



ROC analysis for the technical replication. Luminex data for 100 patients versus 100 healthy controls are shown. 95% confidence intervals highlighted in blue.

Autoantibody reactivity above the cut-off level was identified in 50% of the previously negative-tested SSc patients. The genes encoding for these proteins were found being enriched in pathways of histone modifications and chromatin remodeling suggesting their involvement in epigenetic processes.

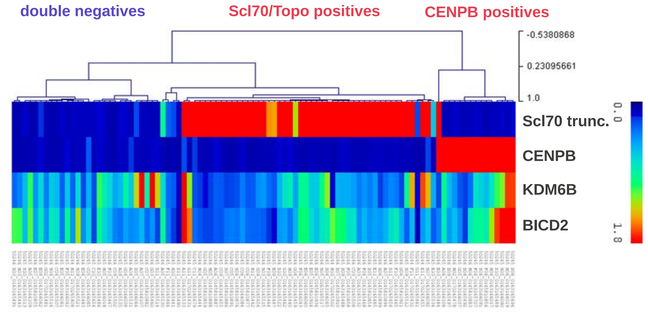


	CENPC1	CENPB	CENPA	BICD2	KDM6B	Scl70FLDia
HC	3	7	3	4	5	3
SSc	44	37	35	26	24	33
Limited	60	62	36	40	18	
Diffuse	34	17	14	21	10	66

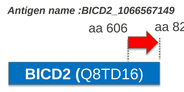
Frequency of autoantibodies against anti-centromere, Scl70, KDM6B and BICD2 in SSc and SSc subgroups.

Conclusion

Using a combination of Luminex bead-arrays for high-throughput autoantibody profiling and complementary Assay development provides an attractive route to discover and verify novel SSc-associated autoantibodies. By measuring 7 antigens the number of autoantibody positive SSc patients increased from 68% to 84%. Interestingly, genes encoding these novel autoantigens have been implicated in epigenetic processes.



- The reactivity profile against the 2 novel antigens partially overlaps with anti-CENPB and anti-Scl70
- Antibodies against the 2 novel antigens are present in double negative patients and potentially provide extra value



The recognized novel biomarker target autoantigens are fragments of larger protein precursors.

The respective KDM6B polypeptide matches the N-terminus of KDM6B, whereas the BICD2 polypeptide matches the corresponding C-terminus of the protein precursor.