High-Resolution PEPperMAP® Epitope Mapping of a Novel Autoimmune Biomarker

Introduction

This report describes the epitope mapping of autoimmune sera against a putative systemic sclerosis (SSc) biomarker identified by Protagen[1] in SSc patient and control samples.

Systemic sclerosis (SSc; also termed scleroderma) is a complex, multisystem rheumatic disease with high mortality and morbidity. Diagnosis of SSc is based on the combination of clinical and/or laboratory features e.g. SSc-related autoantibodies. The most frequently measured autoantibodies (anti-Scl70, anti-centromere like CENPA, anti-RNPIII) are only present in about 60-70% of SSc patients. This highlights the need for additional specific and sensitive diagnostic, prognostic and therapeutic response biomarkers in SSc. Therefore, Protagen applied its SeroTag® process to discover new autoantibodies with high frequency in SSc. The SeroTag® process is an automated bead-based, antigen array on the Luminex FlexMAP3D analyzer for measuring autoantibody reactivities against both established and putative autoantigens. Three putative SSc antigens were identified by Protagen, one of them being KDM6B (Lysine (K)-Specific Demethylase 6B).

To characterize the autoantibody responses against KDM6B, a high resolution epitope mapping with 15mer peptides and peptide-peptide overlap of 14 amino acids was done by PEPperPRINT. In parallel, we mapped the epitopes of centromere protein A (CENPA) specific autoantibodies with an N-terminal main epitope PRRRS identified by Muro et al.[2] We compared 3 SSc patient sera and one healthy control serum and identified well defined polyclonal response with high signal-to-noise ratios against both autoantigens. Due to the high maximum peptide-peptide overlap, we further could identify two N-terminal neighbored epitopes within CENPA with a higher epitope resolution than before. Besides various other epitopes, we also identified a common epitope for the putative SSc antigen KDM6B.

Results

PEPperMAP® Type 1 Epitope Mappings of three human SSc patient sera (SSc1, SSc2, SSc3) and one healthy control serum (HC) were done against antigens CENPA and KDM6B that were translated into 15 aa peptides with maximum peptide-peptide overlaps of 14 aa. The peptide microarrays with the antigen-derived peptides were incubated with the human sera at dilutions of 1:5000 followed by staining with the secondary F(ab´)2 goat anti-human IgG(H+L) conj. DyLight680 antibody and by read-out with a LI-COR Odyssey Imaging System. Quantification of spot intensities and peptide annotation were done with PepSlide® Analyzer.

Sera SSc1, SSc2 and SSc3 showed a strong response with excellent signal-to-noise ratios against epitopes in both antigens CENPA and KDM6B. (Fig. 1).

[1] https://protagenproteinservices.com/

[2] Y. Muro et al., Clin. Exp. Immunol. 2000; 120:218-223

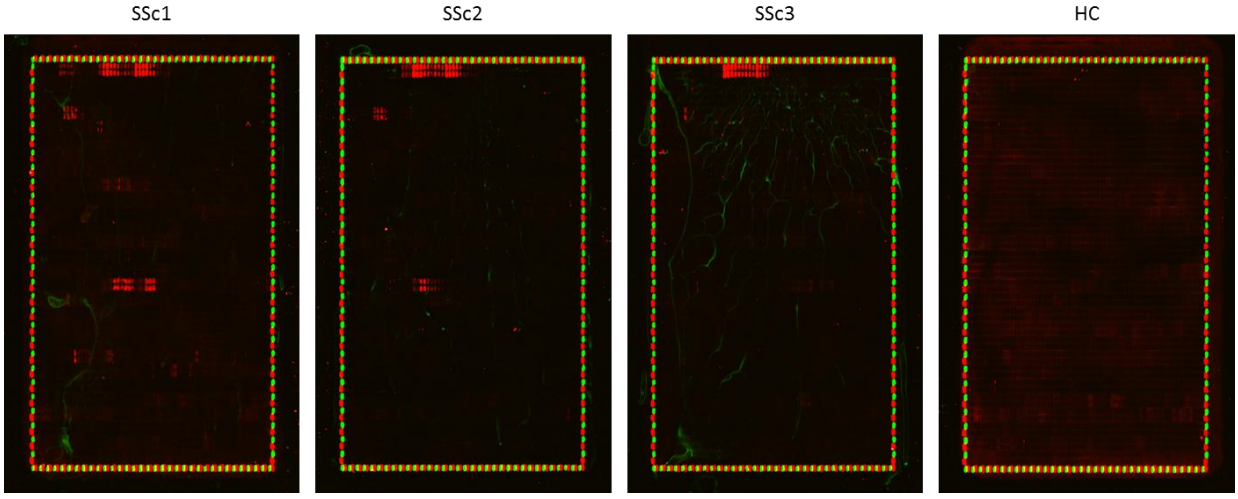

Fig. 1: Peptide arrays were assayed with human sera SSc1, SSc2, SSc3 and HC at dilutions of 1:5000 followed by staining with the secondary antibody and data read-out. Final staining of the HA and Flag control peptides framing the peptide arrays gave rise to the expected and well-defined spot pattern and validated the overall peptide microarray integrity.

Two CENPA epitopes SPSPTPTPGPSR and GPSRRGPSLGAS were observed in all SSc sera as common epitopes with excellent signal-to-noise ratios (Fig. 2).

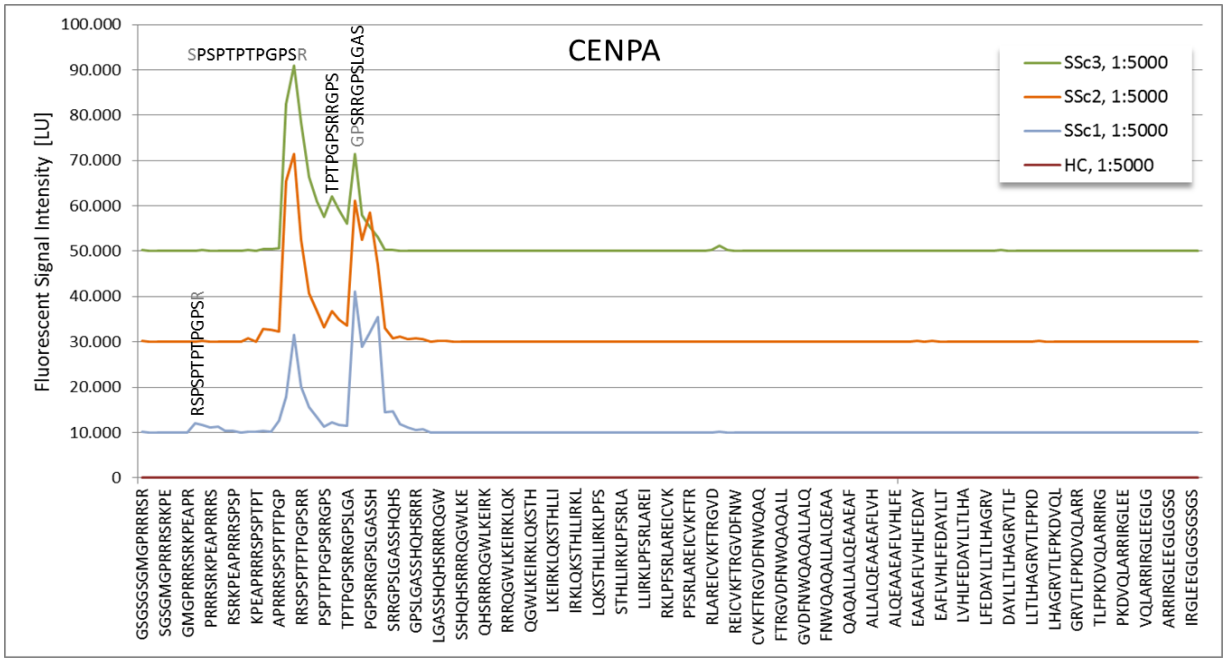

Fig. 2: The intensity plots of the CENPA peptides highlight the strong and widely identical response of sera SSc1, SSc2 and SSc3 with the two main epitopes SPSPTPTPGPSR and GPSRRGPSLGAS and a possible third but overlaid epitope TPTPGPSRRGPS. Serum SSc1 showed a slight difference in the intensity ratio of both main epitopes and gave rise to an additional N-terminal epitope RSPSPTPTPGPSR (intensity plots were leveled to provide a clearer data overview.)

All SSc sera strongly responded against a common epitope LPAPLPPSHGSS within KDM6B. Sera SSc1 and SSc2 further recognized a second epitope SPQPSASSSSQF, which was not recognized by autoantibodies of SSc3 (Fig. 3). As expected, control serum HC did not show any specific reaction with CENPA and KDM6B.

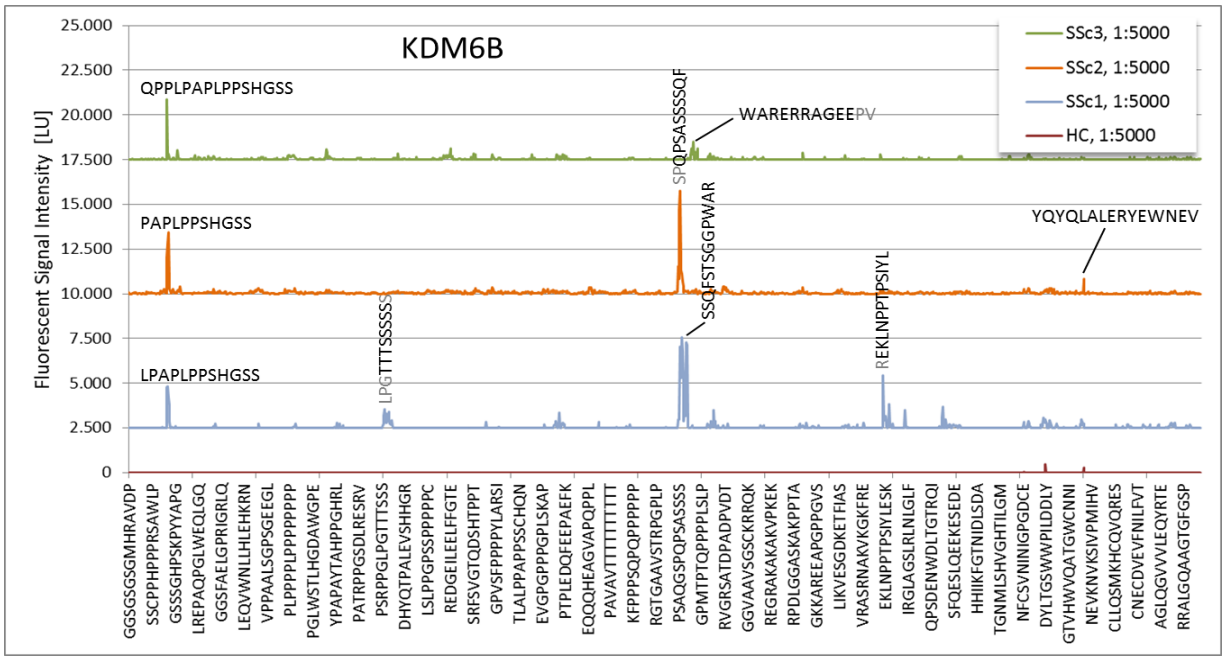

Fig. 3: The intensity plots of the KDM6B peptides highlighted the moderate to strong and in part similar response of sera SSc1, SSc2 and SSc3 against the main epitopes LPAPLPPSHGSS and SPQPSASSSSQF. While SPQPSASSSSQF was only observed in sera SSc1 and SSc2, epitope LPAPLPPSHGSS was common in all SSc serum samples. Other epitopes like REKLNPPTPSIYL or SSQFSTSGGPWAR were only identified in one of the samples (the intensity plots were leveled to provide a clearer data overview.)

Conclusion

Sera SSc1, SSc2 and SSc3 showed a strong response with excellent signal-to-noise ratios against epitopes in both antigens. Two CENPA epitopes as well as one KDM6B epitope were observed in all three serum samples as common epitopes. Other epitopes like RSPSPTPTPGPSR (SSc1) in CENPA or SPQPSASSSSQF (SSc1 and SSc2) were only identified in one or two of the positive sera. In contrast to the excellent signal-to-noise ratios of the positive samples, serum HC only showed only some non-specific background interactions. In accordance with the study of Muro et al.,[2] we observed a clear and very strong autoantibody response against the N-terminus of CENPA. Due to the high epitope resolution, however, we were able to identify at least two neighbored but different epitopes instead of the reported single epitope PRRRS, what underlined the benefit of high resolution PEPperMAP® Type 1 Epitope Mappings.

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