Concise Report

Detection of putative autoantibodies in systemic lupus erythematous using a novel native-conformation protein microarray platform

Lupus
0(0) 1–7

DOI: 10.1177/0961203320959696
journals.sagepub.com/home/lup
SAGE

Anselm Mak[1,2], Nien Yee Kow[1], Nur Hafiza Ismail[3], Nur Diana Anuar[3], Nurul H Rutt[3], Jiacai Cho[1,2], Nurul Shielawati Binti Mohamed Rosli[3] and Bhushan Dharmahidari[4,5]

Abstract

Objective: Conventional immunoassays detect autoantibodies related to systemic lupus erythematosus (SLE) via recognition of epitopes on autoantigens expressed in their denatured rather than native conformational state, casting difficulty in evaluating the genuine pathogenicity of the autoantibodies. We aimed to use a novel high-throughput protein microarray platform to identify autoantibodies against native autoantigens in SLE sera.

Methods: Sera from SLE patients and those of gender-, age-, and ethnicity-matched healthy controls (HC) were screened against more than 1,600 immune-related antigens of native conformation. The relative fluorescent unit readout from post-assay imaging were subjected to bioinformatics pre-processing and composite normalization. A penetrance fold change (pFC) analysis between SLE and HC samples shortlisted 50 autoantigens that were subjected to an unsupervised cluster analysis. Correlations between the pFC of putative autoantigens and clinical parameters including SLE disease activity index (SLEDAI-2K) and recent SLE flares were explored.

Results: 381 autoantigens were identified when 15 SLE and 15 HC serum samples were compared. The top 20 autoantigens which elicited autoantibody responses in SLE sera filtered based on the highest pFC were further analyzed. Autoantigens which the putative autoantibodies reacted against are those involved in chromatin organization such as DEK, regulation of transcription activity including REOX4 and ELF4, and negative regulation of NF_kB activity such as TRIB3. Additionally, the pFC of these autoantibodies significantly and positively correlated with SLEDAI-2K and recent SLE flares.

Conclusion: A high-throughput protein microarray platform allows detection and quantification of putative lupus-related autoantibodies which are of potential pathophysiological and prognostic significance in SLE patients.

Keywords

Autoantibody, lupus, high-throughput, microarrays, proteins

Date received: 10 June 2020; accepted: 28 August 2020

Introduction

Systemic lupus erythematosus is a prototype autoimmune disease characterized by multifactorial aetiologies and a plethora of autoantibodies which induce immune-complex mediated inflammation multi-systemically.[1,2] Detection of the presence of autoantibodies and their relationships with specific clinical manifestations is one of the fundamental strategies to further

[1]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[2]Division of Rheumatology, University Medicine Cluster, National University Health System, Singapore, Singapore
[3]Sengenics Corporation Pte Ltd, Sengenics, Singapore, Singapore
[4]Department of Physiology, National University of Singapore, Singapore, Singapore
[5]Immunology Program, National University of Singapore, Singapore, Singapore

Corresponding author:
Anselm Mak, Division of Rheumatology, Department of Medicine, 1E Kent Ridge Road, Level 10, NUHS Tower Block, Singapore 119228, Singapore.
Email: mdcam@nus.edu.sg

understand the pathogenesis of SLE and discover clinically useful diagnostic and prognostic biomarkers for the autoimmune condition.

Conventional assays including counter protein electrophoresis, western blot assay, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay and the contemporary multiplex platforms identify and quantify target proteins including autoantibodies by recognizing the epitopes on the autoantigens precoated on the respective assay platforms. These conventional immunoassays often utilize purified proteins to fabricate antigens on the detection surface.[3] Protein purification involves rigorous processes that utilize ionic detergents to separate the protein of interest from other cellular residues that denature native protein conformation, potentially contributing to the loss of conformational epitopes. Since more than 90% of antibody epitopes are discontinuous and presented on the solvent-exposed surface of the target antigens,[4] the interaction between antibodies with correctly-folded antigens in native conformation will enhance the specificity and sensitivity of autoantibody detection. Direct ELISA, which is commonly used for screening and validation of disease biomarkers, is often influenced by avidity and cross reactivity issues.[5,6] To circumvent this problem, sandwich and competitive ELISA which utilize 8pG cells that stably express poly-protein G on the cell membrane coated on the microplate to capture antibody are the alternatives because the absence of additional protein purification procedures in these assays will enhance their detection sensitivity.[5]

Recently, a novel high-throughput microarray protein assay (Sengenics KREX^{TM} Immunome^{TM} Protein Microarray) that facilitates biomarker identification has been developed. This technique can detect a vast array of antibodies in their native 3D-conformational structures with small volume serum samples (as little as 10μl) based on a patented recombinant protein expression technique.[7] Such technique involves the utilization of the biotin carboxyl carrier protein (BCCP) folding marker that is cloned in-frame with the gene encoding the protein of interest. BCCP is fused either to the N- or C-terminal of the proteins of interest. Full-length proteins are expressed as fusions to the BCCP folding marker that are biotinylated *in vivo* by host cell biotin ligase only when the proteins are correctly folded. Misfolded proteins, conversely, drive the co-translational misfolding of BCCP, rending it catalytically inactive and unable to be biotinylated. As a result, misfolded proteins are incapable of being immobilized onto a streptavidin-coated solid platform where 3D conformation autoantibodies are bound, detected and quantified (Figure 1(a)). Compared to indirect ELISA, the Sengenics KREM^{TM} Immunome^{TM} protein microarray adopts the direct ELISA principle which is technically simpler and with greater multiplexity. The Sengenics platform comprises more than 1,600 full-length and natively-folded proteins (antigens) that represent different classes and various types of immune-related proteins including cytokines, interleukins (IL) and transcription factors, making it a favorable platform to detect and quantify autoantibodies and discover biomarkers relevant to the pathophysiology of SLE.

Methods

Adult (age ≥ 21) patients who fulfilled the 1997 or 2012 classification criteria for SLE[8,9] were recruited at the Lupus Clinic, National University Medical Centre, National University Hospital (NUH), and Singapore. Healthy controls (HC) who were staff and their relatives of the Lupus Clinic and matched for age, gender and ethnicity were recruited for comparison. Demographic data of the SLE patients and HC were collected through clinical interview. Clinical information including the disease duration of SLE, medication use, SLE disease activity as assessed with the use of the SLE Disease Activity Index-2K (SLEDAI-2K),[10] SLE flare within 4 weeks prior to recruitment for this study, SLE-related damage evaluated by the Systemic Lupus Erythematosus International Collaborating Clinic/ACR damage index (SLICC/ACR DI).[11] Laboratory parameters including serum creatinine, complement (C3 and C4) and anti-dsDNA levels, estimated glomerular filtration rate (eGFR) and autoantibody profile including antinuclear antibody (ANA), anti-dsDNA and anti-extractable nuclear antigen antibodies (anti-ENA) including anti-Ro, Sm and RNP were assayed at the standard hospital laboratory of the NUH.

Three milliliters of peripheral blood were obtained from each subject via venipuncture and were centrifuged at 13,000*g* for 15 minutes to obtain the sera that were stored at –80°C for subsequent analyses. The sera were diluted with phosphate buffered saline at 1:400 and were screened for more than 1,600 proteins on the Sengenics KREX^{TM} Immunome^{TM} platform, followed by image analyses, data extraction and pre-processing, quality control (QC) and composite normalization using a previously published method.[12] The levels of autoantibodies against each protein on the arrays are represented as relative fluorescent unit (RFU). For each protein, the RFU for SLE patients versus HC were compared by a penetrance fold change (pFC) analysis as per the protocol established by Sengenics. In brief, individual fold changes (IFC) of SLE patients and HC were calculated by dividing the RFU value for each protein in each sample by the mean of the RFU value of each protein across all

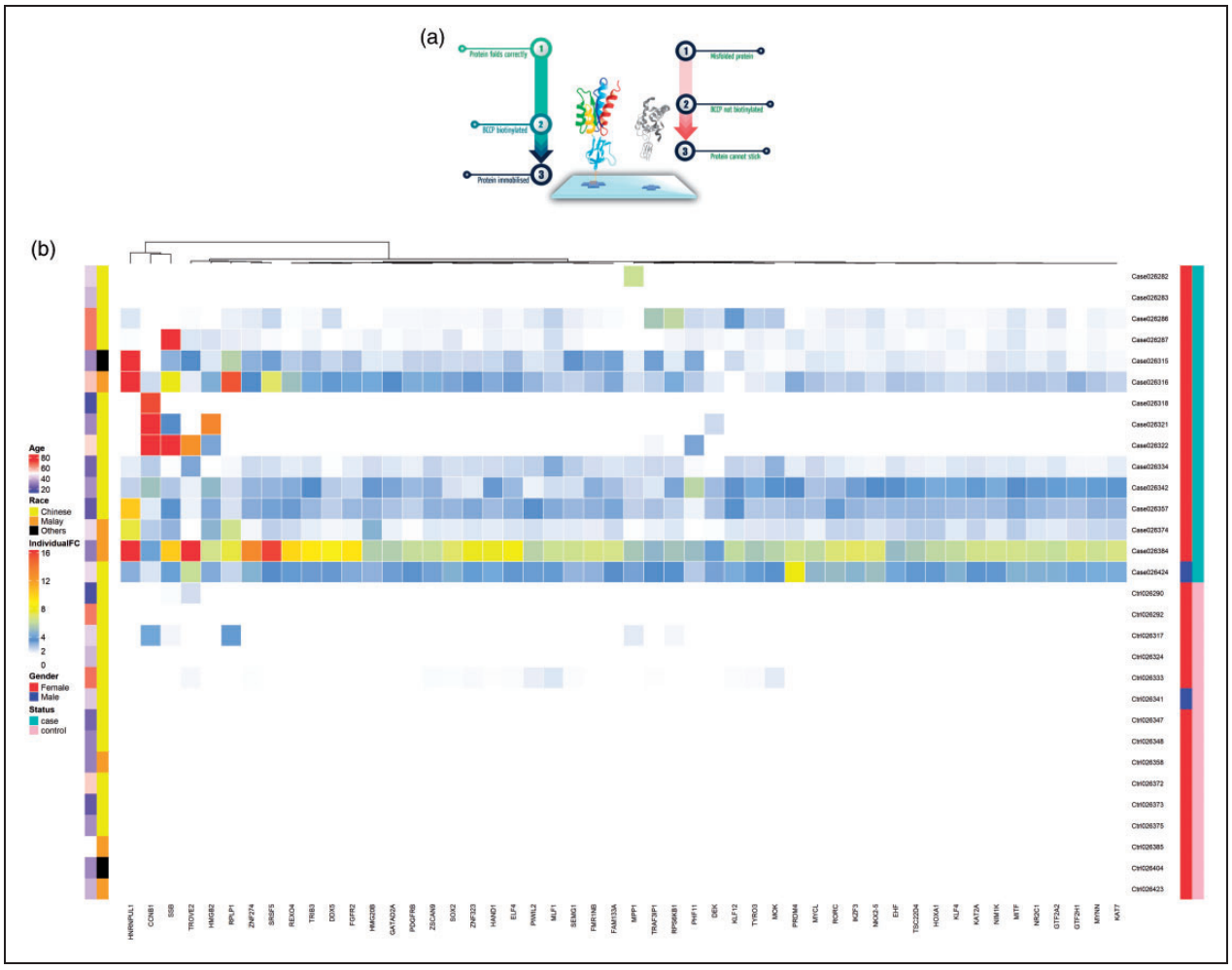

Figure 1. The principles of high-throughput protein array technology and statistical analyses of the presence and levels of novel serum autoantibodies. (a) The principle of autoantigen (in native 3-dimensional conformation) detection by the Sengenics KREX™ Immunome™ platform high-throughput protein-array technology. Only correctly folded proteins (autoantigens potentially related to SLE) of their native 3D-conformation can biotinylate BCCP and immobilize proteins for stable and valid detection. (b) Heat map representing the top 50 significant autoantigens detected by autoantibodies from 15 SLE patients across the SLE and HC sera.

the HC samples (i.e. background threshold). pFC values were then calculated by obtaining the mean IFC across all patients which pass the individual fold change threshold of ≥ 2. The penetrance frequencies (number of SLE and HC samples with IFC ≥ 2 folds) of both SLE and HC sera were then determined for each protein. Putative biomarkers were identified and ranked based on the following criteria: i) pFC of SLE patient ≥ 2 and ii), % frequency of SLE patient $\geq 60\%$. With the use of the top 50 antigens, an unsupervised cluster analysis was performed across all the samples using a hierarchical clustering method; that is, the Ward's method and distance calculated based on Euclidean distance.[13]

Values were expressed as mean $\pm$ standard deviation (SD) unless otherwise specified. Continuous and discrete variables between the SLE and HC groups were compared by using Mann-Whitney U test and χ^2 test, respectively. Correlations between IFC of each protein and demographic and clinical parameters were explored by bivariate correlation analyses. All statistical analyses were performed with SPSS (SPSS version 22.0, Chicago, IL, USA). A two-tailed p value of <0.05 was considered statistically significant. Written informed consents were obtained from all subjects before recruitment. The study was approved by the NHG Domain Specific Review Board (Domain E, DSRB number 2017/00324).

Results

Fifteen SLE patients and 15 gender-, age-, and ethnicity-matched HC were recruited for this study, their sera and 2 pooled normal sera (Sengenics internal

QC samples) were screened for autoantibodies on the Sengenics KREX™ Immunome™ platform. Table 1 summarizes the demographic and clinical information of the recruited subjects.

Antibody responses against 318 autoantigens were identified in SLE and HC sera. Hierarchical clustering of the top 50 significant antigens across the SLE and HC sera revealed clear differences in autoantibody titers that corresponded to the pFC analyses (see Figure 1(b) for heat map presentation). Based on the penetrance frequency, autoantibodies against antigens SSB, GATAD2A, DEK, HNRNPUL1, CCNB1 and TROVE2 ranked highest amongst the top 20 autoantibodies identified (Supplementary Table 1, Table 1). When assessed according to the pFC of individual antigens, autoantibody response against antigens HNRNPUL1, CCNB1, SSB, TROVE2, HMGB2, RPLP1 and SRSF5 were found to be particularly high in a number of SLE patients (Figure 1(b)).

Amongst the top 20 autoantigens with significant responses identified (Table 1), correlation analyses were performed to explore their relationship with various clinical parameters (Supplementary Table 1). High pFC of a number of putative biomarkers were significantly associated with active disease in terms of higher SLE disease activity and the presence of recent SLE flares. There were highly expressed autoantibodies against 9 antigens which include DEK, ELF4, FGRF2, HMF20B, HMGB2, HNRUPUL1, MITF, REOX4 and TRIB3 were significantly associated with higher SLEDAI-2K. Autoantibodies against CCNB1 and HMGB2 were inversely associated with serum C4 level, indicating their relationships with higher SLE disease activity (Supplementary Table 1). The pFC of antibodies against 2 closely-linked proteins, HMG20B and HMGB2, were also significantly associated with higher anti-dsDNA level and daily prednisolone dose apart from SLEDAI-2K. Nevertheless, significant relationships were found between daily prednisolone dose and clinical parameters of active SLE including high SLEDAI-2K and recent SLE flares, high anti-dsDNA level and low C3 and C4 levels (see footnote of Supplementary Table 1). Patients who had flare within 4 weeks when their serum samples were obtained had higher pFC against GATAD2A, HAND1, DEKKLF12, PRDM4, REOX4 and TRIB4.

As for sub-analyses pertaining to different anti-ENA antibody statuses, patients who were positive for anti-Ro and anti-Sm had significantly higher levels of anti-TROVE2 and anti-SSB antibodies, respectively. Such relationship is not surprising as TROVE2 was apparently the same protein as Ro60 as found in patients with primary Sjögren's syndrome.[14] In addition, putative biomarkers including HNRNPUL1, RPLP1, REOX4, SRSF5 and SSB were associated with patients who were anti-RNP positive (Supplementary Table 2). Supplementary Table 3 summarizes the GenBank reference and accession number for each target of the Sengenics™ protein microarray platform.

Discussion

This novel high-throughput protein microarray platform allowed identification and quantification of putative autoantigens/biomarker that elicit autoantibody response in patients with SLE. Intriguingly, a number of these biomarkers that were found to be significantly related to SLE disease activity and recent disease flares recognize antigens that function to regulate the cell cycle and transcription and translation of genes. For instance, antigens including DEK which has previously been found to be elevated in pediatric patients with juvenile inflammatory arthritis and SLE,[15,16] as well as CCNB1 are involved in chromatin organization,[17] CCNB1 and HMG20B control cell cycle at the G2/M transition.[18] HMGB2 encodes non-histone chromosomal HBG protein family that promotes DNA flexibility by facilitating cooperative interaction between cis-acting protein and involving the final ligation step in DNA double strand break repair and V(D)J recombination.[19] GATAD2A is a transcriptional repressor that enhances MBD2-mediated repression.[20] Potential loss of MBD2-mediated repression with an antibody against GATAD2A might lead to the loss of speckled nuclear localization. As for the putative autoantibodies which recognize proteins that are involved in the regulation of DNA transcription and RNA translation, we found antibodies against ELF4 that promotes transcription of an activator that activates the *CSF2*, *IL-8* and *PRF1* genes which affect NK cell development and induce cell cycle arrest in naïve $CD8^+$ T cells,[21] REXO4 that is involved in nucleic acid binding and DNA-binding transcription factor activities,[22] PRDM4 that encodes a transcription factor that affects cell differentiation and HNRNPUL1 that is involved in nucleocytoplasmic RNA transport.[23] Based on the findings of this study, it is imperative to strengthen the research direction of investigating the impact of lupus-related autoantibodies on the cellular physiology, particularly how these autoantibodies affect the cell cycle and its impact on 1) leucocyte function as a result of modification of DNA transcription and RNA translation, and 2) organs where the rate of cell division is relatively low such as the kidneys and brain, leading to irreversible organ damage. Furthermore, whether these novel autoantibodies are functionally pathogenic, protective or neutral towards the pathophysiology of SLE require to be further elucidated by mechanistic studies because

Table 1. Demographic, clinical information and penetrance fold change of autoantibodies in patients with systemic lupus erythematosus and healthy subjects.

	SLE	Healthy control	P value
	Mean ± SD (range) Number (%)		
Age	42.07±12.5	41.40 ± 12.3	0.884
Gender, female	14 (93.3)	14 (93.3)	1.0
Ethnicity			1.0
Chinese	11 (73.3)	11 (73.3)	1.0
Malay	3 (20)	3 (20)	
Other	1 (6.7)	1 (6.7)	
Duration of SLE, months	155.53 ± 93.7	NA	NA
Serum creatinine, μmol/L	103.33 ± 166.9	NA	NA
eGFR, ml/min	99.33 ± 33.1	NA	NA
Spot urine protein, gm/day	0.17 ± 0.1	NA	NA
ANA positivity[a]	15 (100)	NA	NA
Anti-Ro positivity[b]	7 (46.7)	NA	NA
Anti-RNP positivity[b]	6 (40)	NA	NA
Anti-Sm positivity[b]	4 (26.7)	NA	NA
Serum C3, U/L	87.54 ± 27.3	NA	NA
Serum C4, U/L	20.15 ± 15.1	NA	NA
Serum anti-dsDNA, IU/L (range)	220.0 ± 254.0 (3–800)	NA	NA
SLEDAI-2K, unit (range)	2.27 ± 2.6 (0–8)	NA	NA
SLICC/ACR DI, unit (range)	0.53 ± 1.1 (0–3)	NA	NA
Prednisolone, (range), mg/day	2.73 ± (0–12.5)	NA	NA
Hydroxychloroquine, (range), mg/day	213.33 ± 124.6 (0–400)	NA	NA
Serum autoantibody, penetrance fold change (pFC)[c]			
Anti-SSB	9.78 ± 18.8	1.0 ± 0.6	0.092
Anti-GATAD2A	2.57 ± 1.3	1.0 ± 0.4	<0.001
Anti-DEK	2.36 ± 1.0	1.0 ± 0.5	<0.001
Anti-HNRNPUL1	10.95 ± 19.1	1.0 ± 0.5	0.063
Anti-CCNB1	9.07 ± 18.3	1.0 ± 1.1	0.11
Anti-TROVE2	4.39 ± 5.9	1.0 ± 0.7	0.045
Anti-HMGB2	3.91 ± 3.1	1.0 ± 0.5	0.003
Anti-RPLP1	3.66 ± 3.9	1.0 ± 0.9	0.021
Anti-SRSF5	3.61 ± 3.7	1.0 ± 0.4	0.017
Anti-ZNF274	3.21 ± 2.6	1.0 ± 0.5	0.012
Anti-REOX4	3.08 ± 2.3	1.0 ± 0.5	0.003
Anti-TRIB3	2.91 ± 2.1	1.0 ± 0.4	0.004
Anti-PRDM4	2.88 ± 2.1	1.0 ± 0.5	0.004
Anti-RPS6KB1	2.81 ± 1.7	1.0 ± 0.6	0.001
Anti-ELF4	2.85 ± 1.9	1.0 ± 0.5	0.002
Anti-HMG20B	2.79 ± 1.6	1.0 ± 0.5	0.001
Anti-HAND1	2.81 ± 1.7	1.0 ± 0.5	0.001
Anti-FGFR2	2.75 ± 2.1	1.0 ± 0.5	0.007
Anti-KLF12	2.87 ± 1.5	1.0 ± 0.5	<0.001
Anti-MITF	2.81 ± 1.6	1.0 ± 0.5	0.001

Abbreviations: SLE; systemic lupus erythematosus, NA; not applicable, eGFR; estimated glomerular filtrate rate (based on CKD-EPI equation using standard creatinine), ANA; antinuclear antibody, anti-dsDNA; anti-double-stranded DNA antibody, anti-RNP; anti-ribonucleoprotein antibody, SLEDAI-2K; Systemic Lupus Erythematosus Disease Activity Index-2K, SLICC/ACR DI; Systemic Lupus Erythematosus International Collaborating Clinics/American College of Rheumatology Damage Index, Anti-GATAD2; anti-transcriptional repressor p66-alpha, Anti-DEK; anti-protein DEK, Anti-HNRNPUL1; anti-heterogeneous nuclear ribonucleoprotein U-like protein, Anti-CCNB1; anti-G2/mitotic-specific-cyclin-B1, Anti-TROVE2; anti-60kDa SS-A/Ro ribonucleoprotein, Anti-HMGB2; anti-high mobility group protein B2, Anti-RPLP1; anti-60S acidic ribosomal protein P1, Anti-SRSF5; anti-serine/arginine-rich splicing factor 5, Anti-ZNF274; anti-neurotrophin receptor-interacting factor homolog, Anti-REOX4, anti-RNA exonuclease 4; Anti-TRIB3, anti-tribbles homolog 3; Anti-PRDM4, anti-PR domain zinc finger protein 4; Anti-RPS6KB1, anti-ribosomal protein S6 kinase beta-1; Anti-ELF4, anti-ETS-related transcription factor Elf-4; Anti-HMG20B, anti-SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1-related; Anti-HAND1, anti-heart- and neural crest derivatives-expressed protein 1; Anti-FGFR2, anti-fibroblast growth factor receptor 2; Anti-KLF12, anti-Krueppl-like factor 12; Anti-MITF, anti-microphthalmia-associated transcription factor

[a]Cut-off ANA titre >1:80 (conducted by in-house enzyme-linked immunosorbent assay).

[b]Conducted by enzyme-linked immunosorbent assay (Euroimmun®). Values are expressed as ratios of optical absorbance of patient's samples versus that of commercial mixed healthy serum samples. Reference values: negative if absorbance ratio was <1.0, weakly positive if absorbance ratio was between 1.0 and 2.0, positive if absorbance ratio was between >2.0–5.0, strongly positive if absorbance ratio was >5.0. In Table 1, serum anti-Ro, anti-RNP and anti-Sm antibodies were considered positive if the absorbance ratio was >2.0.

[c]pFC of SLE patients were normalized to those of the healthy subjects.

of the multifaceted functions of antibodies in various autoimmune diseases and infections.[24,25]

Limitations exist in this study. The limited budget allocated to this pilot study only allowed a sample size of 30 subjects due to the high cost of this novel protein microarray technology. Therefore, we set out to recruit 15 SLE patients with a range of SLE disease activity and meticulously match them with healthy subjects demographically, aiming to mitigate the impact of these confounders on autoantibody detection. The small sample size disallowed multivariate adjustments for confounders such as daily prednisolone dose. Nevertheless, the perfectly consistent direction of the associations between the pFC of the putative autoantibodies and the clinical indicators of active diseases implies the validity of our data. While another study of a larger sample size of SLE patients that adopted a similar protein microarray platform was published, the aim of that study was to identify autoantibody clusters with respect to the clinical presentation and serological patterns.[12] Our study, on the other hand, identified putative lupus-related autoantibodies and their associations with active disease and recent disease flares. Owing to the limited size of our cohort and the breadth of the autoantibodies tested by the hospital ELISA system, a comprehensive repertoire and pathological implications of the SLE-related autoantibodies detected by this novel protein microarray platform remain to be established. Additionally, while an in-depth review described around 180 autoantigens in SLE patients,[26] our protein microarray platform discovered more as-yet unreported autoantibodies that correlated with active disease and recent disease flares. Lastly, type 1 error would have been encountered when comparisons were made statistically. Nevertheless, the signals detected in this preliminary study will invariably provide a foundation for sample size calculation when we are planning for a validation study. In conclusion, this pilot study has laid the foundation of further prospective evaluation of the potential pathogenic roles of these putative lupus-related autoantibodies by involving a larger sample size and in-depth evaluation of the impact of these autoantibodies on interruption of certain cellular mechanisms, particularly in the realms of chromatin arrangement and cell division checkpoints, together with those aspects that have been actively evaluated contemporarily such as gene transcription and translation and post-translational modifications in relation to the induction and aggravation of active SLE. In addition, the potential of this novel protein microarray platform in combination with omics and functional analyses would pave the way to identify personalized diagnostic and prognostic biomarkers and therapeutic targets in patients with SLE.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the MOE Tier 1 grant (Grant number: R-172-000-410-114).

ORCID iD

Anselm Mak https://orcid.org/0000-0002-4688-7829

Supplemental material

Supplemental material for this article is available online.

References

1. Kwon YC, Chun S, Kim K and Mak A. Update on the genetics of systemic lupus erythematosus: genome-wide association studies and beyond. *Cells* 2019; 8: 1180.
2. Tan MKX, Heng TYJ and Mak A. The potential use of metformin, dipyridamole, N-acetylcysteine and statins as adjunctive therapy for systemic lupus erythematosus. *Cells* 2019; 8: 323.
3. Sun SQ, Guo HC, Sun DH, et al. Development and validation of an ELISA using a protein encoded by ORF2 antigenic domain of porcine circovirus type 2. *Virol J* 2010; 7: 274.
4. Van Regenmortel MH. Immunoinformatics may lead to a reappraisal of the nature of B cell epitopes and of the feasibility of synthetic peptide vaccines. *J Mol Recognit* 2006; 19: 183–187.
5. Chen YJ, Chen M, Hsieh YC, et al. Development of a highly sensitive enzyme-linked immunosorbent assay (ELISA) through use of poly-protein G-expressing cell-based microplates. *Sci Rep* 2018; 8: 17868.
6. Garcia HH, Castillo Y, Gonzales I, et al. Low sensitivity and frequent cross-reactions in commercially available antibody detection ELISA assays for Taenia solium cysticercosis. *Trop Med Int Health* 2018; 23: 101–105.4
7. Sengenics UK Ltd. Sengenics KREX presentation, www.sengenics.com/wp-content/uploads/2018/04/US8999897.pdf
8. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
9. Petri M, Orbai AM, Alarcón GS, et al. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012; 64: 2677–2686.
10. Parodis I, Emamikia S, Gomez A, Gunnarsson I, van Vollenhoven RF and Chatzidionysiou K. Clinical SLEDAI-2K zero may be a pragmatic outcome measure in SLE studies. *Expert Opin Biol Ther* 2019; 19: 157–168.

11. Stoll T, Seifert B and Isenberg DA. SLICC/ACR damage index is valid, and renal and pulmonary organ scores are predictors of severe outcome in patients with systemic lupus erythematosus. *Br J Rheumatol* 1996; 35: 248–254.
12. Lewis MJ, Mcandrew MB, Wheeler C, et al. Autoantibodies targeting TLR and SMAD pathways de fi ne new subgroups in systemic lupus erythematosus. *J Autoimmun* 2018; 91: 1–12.
13. Vogt W and Nagel D. Cluster analysis in diagnosis. *Clin Chem* 1992; 38: 182–198.
14. Gourzi VC, Kapsogeorgou EK, Kyriakidis NC and Tzioufas AG. Study of microRNAs (miRNAs) that are predicted to target the autoantigens Ro/SSA and La/SSB in primary Sjögren's syndrome. *Clin Exp Immunol* 2015; 182: 14–22.
15. Dong X, Wang J, Kabir FN, et al. Autoantibodies to DEK oncoprotein in human inflammatory disease. *Arthritis Rheum* 2000; 43: 85–93.
16. Mor-Vaknin N, Rivas M, Legendre M, et al. High levels of DEK autoantibodies in sera of patients with polyarticular juvenile idiopathic arthritis and with early disease flares following cessation of anti-tumor necrosis factor therapy. *Arthritis Rheumatol* 2018; 70: 594–605.
17. Schnittger A and De Veylder L. The dual face of cyclin B1. *Trends Plant Sci* 2018; 23: 475–478.
18. Lee M, Daniels MJ, Garnett MJ and Venkitaraman AR. A mitotic function for the high-mobility group protein HMG20b regulated by its interaction with the BRC repeats of the BRCA2 tumor suppressor. *Oncogene* 2011; 30: 3360–3369.
19. Paull TT, Haykinson MJ and Johnson RC. The nonspecific DNA-binding and -bending proteins HMG1 and HMG2 promote the assembly of complex nucleoprotein structures. *Genes Dev* 1993; 7: 1521–1534.
20. Walavalkar NM, Gordon N and Williams DC Jr. Unique features of the anti-parallel, heterodimeric coiled-coil interaction between methyl-cytosine binding domain 2 (MBD2) homologues and GATA zinc finger domain containing 2A (GATAD2A/p66α). *J Biol Chem* 2013; 288: 3419–3427.
21. Yamada T, Park CS, Mamonkin M and Lacorazza HD. Transcription factor ELF4 controls the proliferation and homing of CD8+ T cells via the Krüppel-like factors KLF4 and KLF2. *Nat Immunol* 2009; 10: 618–626.
22. Krishnamurthy N, Ngam CR, Berdis AJ and Montano MM. The exonuclease activity of hPMC2 is required for transcriptional regulation of the QR gene and repair of estrogen-induced abasic sites. *Oncogene* 2011; 30: 4731–4739.
23. Hong Z, Jiang J, Ma J, Dai S, Xu T, Li H and Yasui A. The role of hnRPUL1 involved in DNA damage response is related to PARP1. *PLoS One* 2013; 8: e60208.
24. Casadevall A and Pirofski LA. A new synthesis for antibody-mediated immunity. *Nat Immunol* 2011; 13: 21–28.
25. Fritzler MJ. Toward a new autoantibody diagnostic orthodoxy: understanding the bad, good and indifferent. *Auto Immun Highlights* 2012; 3: 51–58.
26. Yaniv G, Twig G, Shor DB, et al. A volcanic explosion of autoantibodies in systemic lupus erythematosus: a diversity of 180 different antibodies found in SLE patients. *Autoimmun Rev* 2015; 14: 75–79.