Autoantibody Discovery using a Novel Microarray of Functional Proteins

Technology background

Recombinant protein expression is a fundamental technique that underpins clinical diagnostics, drug discovery and screening, vaccine development and pure research for elucidating mechanisms of disease development and progression. However, high-throughput production of correctly folded and functional, full-length human proteins has a very high failure rate. Protein folding is a highly complex process requiring a combination of an aqueous environment, chaperones, post-translational modifications and the formation of multimeric structures held together by covalent bonds. Any deviation from the correct sequence of events can result in a misfolded protein. Loss of protein function is directly linked to misfolding. Use of misfolded proteins in downstream assays and interactions studies can result in identification of false positive biomarkers.

The Sengenics KREX™ technology utilises the biotin carboxyl carrier protein (BCCP) as a folding marker and solubility enhancer which results in high-throughput expression of full-length, correctly folded and functional proteins. BCCP-protein fusions are capable of being biotinylated either *in vivo* or *in vitro*, allowing the use of highly specific biotin-streptavidin interaction for surface capture. As biotinylated proteins bound to a streptavidin-coated surface show negligible dissociation, this interaction therefore provides a vastly superior means for tethering proteins to a planar surface and is ideal for applications such as protein microarrays, glass micro-titer plates, SPR and bead-based assays.

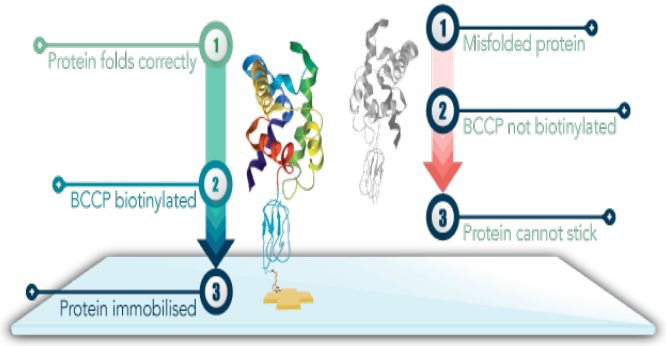

Figure 1. The BCCP folding marker acts as a marker for correctly folded proteins. Proteins will be immobilised on the array only when they are properly folded and biotinylated on the BCCP folding marker.

Introduction

In addition to producing antibodies against foreign molecules, the immune system generates antibodies to self-antigens ("autoantibodies") in response to many pathological processes. Autoantibodies have several properties which make them excellent indicators of disease and their detection forms the basis of many *in vitro* diagnostic tests. It is believed that autoantibodies are generated through over-expression, mutation, release of proteins from damaged tissues, mis-folding or mis-presentation of proteins which leads to their recognition by the immune system.

Unlike other serological targets, autoantibodies are stable, highly specific, easily purified from serum, and are readily detectable with well-validated secondary reagents. Due to their inherent amplification within the immune system, autoantibodies are relatively abundant and are easily measured, making them ideal for early diagnosis of disease.

Leveraging the KREX™ technology, we have developed a fully quantitative protein microarray platform which affords the simultaneous screening of over thousands of functional proteins for various medical and therapeutic proteomics applications. All arrayed proteins are assayed simultaneously under identical conditions resulting in quantitative and genuinely comparative data. It is a highly reproducible, miniaturised assay platform for systematic, high-throughput studies of protein function.

TECHNICAL PERFORMANCE

- Expression of correctly folded and functional proteins with a 98% success rate.
- Fully quantitative clinical-grade assay metrics. Dynamic range is linear up to five orders of magnitude.
- Excellent reproducibility and precision with a mean CV% below 4% between replica spots.
- Highly sensitive with a limit of detection of approximately 1:1,000,000 serum dilution and autoantibody titer of 190 pg/mL.
- Exceptional specificity and accuracy: non-specific binding eliminated as all proteins are immobilised as functional and correctly folded BCCP-fusions.

TYPES OF DISEASES	BIOMARKERS IDENTIFIED
Cancer	Protective
Autoimmune Diseases	Diagnostic
Neurodegenerative Diseases	Screening
Infectious Diseases	Therapeutics

This platform has been successfully used to identify predictive, pathologic and protective biomarkers for cancers, autoimmune, neurodegenerative and infectious diseases. The autoantibody assay protocol is summarised in Figure 2. For each protein, interactions are measured in the form of relative fluorescence units (RFU) using any open format Microarray Scanner at 10µm resolution. Each image is then saved as a 16-bit TIFF file (Figure 3).

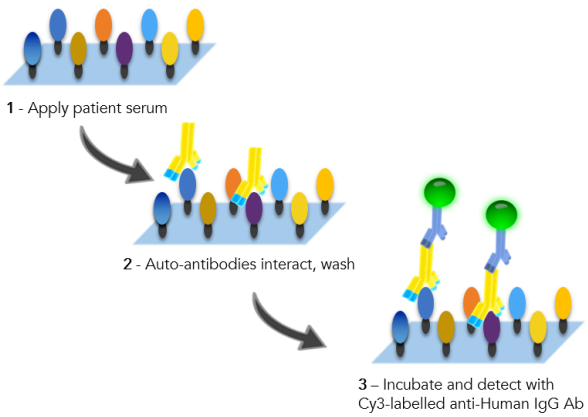

Figure 2. Summary of the autoantibody biomarker discovery protocol.

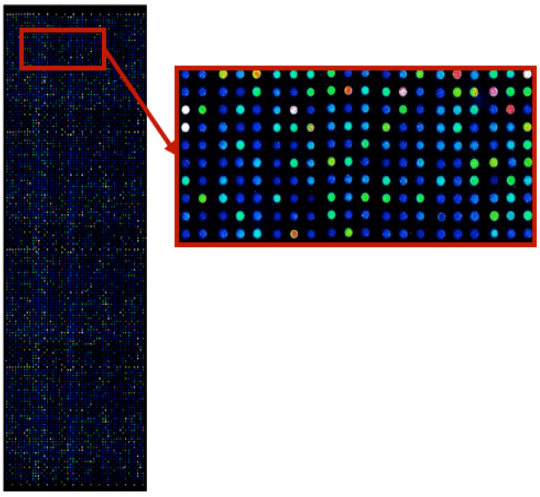

Figure 3. An example tiff. image of the Sengennics 1600+ Immunome™ protein array.

Case studies

1. **Autoantibody Biomarker Discovery for Prostate Cancer using our miniature protein microarray platform; CT100+ array.** *(Adeola, H, et. al., 2016)*

This study aimed to elucidate the role of 123 tumour associated antigens (TAAs) using the Sengenics microarray platform in blood samples from 20 PCa, 32 benign prostatic hyperplasia (BPH) and 15 disease control (DC) cohorts.

Results

Linear quantitation showed four antigens, GAGE1, ROPN1, SPANXA1 and PRKCZ having higher autoantibody titres in PCa serum as compared to BPH where MAGEB1 and PRKCZ were highly expressed.

b.

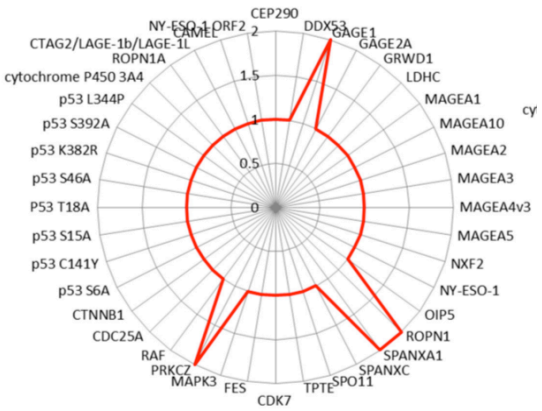

Figure 3. Radar plot showing GAGE1, ROPN1A, SPANXA1 and PRKCZ was highly expressed in PCa.

2. **Study of Neurodegenerative Diseases using the Sengenics 1600+ Immunome Protein Microarray** *(Suwarnalata, G, et. al., 2016)*

Helicobacter pylori (H.pylori) positivity has been associated with greater motor severity in Parkinson's disease (PD). To investigate this association, 30 H. pylori-seropositive PD samples were designated as case and 30 age- and gender-matched H. pylori-seronegative PD samples were used as controls.

Results

This study identified 13 significant autoantibodies based on ranking using the Sengenics penetrance fold change analysis method. Amongst these elevated autoantibodies in H. pylori-seropositive PD, NFIA, PDGFB and eIFA3 have previously been identified as essential proteins involved in neurological function.

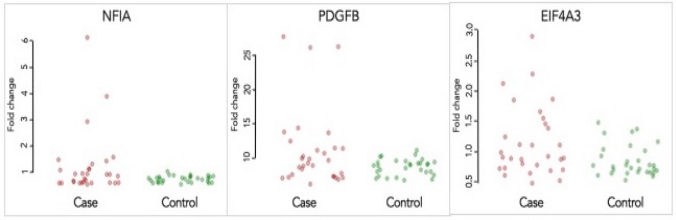

Figure 4. Fold changes between H. pylori-seropositive PD samples (Case) and H. pylori-seronegative (Control) groups for autoantibodies showing higher activity in the case group.

3. **Autoantibody profiling in Infectious Diseases using the Sengenics 1600+ Immunome Protein Microarray** *(Liew, J., et. al., 2015)*

A total number of 22 serum and plasma samples were collected from 11 patients with PCR and microscopically confirmed plasmodium knowlesi malaria infection and 11 age- and gender-matched, healthy individuals (n = 11) who are negative for malaria infection. A comparison analysis of data generated from the protein microarray experiment between these 2 cohorts was performed for the identification of potential autoantibody biomarkers.

Results

This study identified 24 antigens with high reactivity with serum antibodies which are involved in inflammatory processes in the host. These antigens could serve as potential biomarkers for cases of asymptomatic malaria and mild malaria or predictive markers for severe malaria.

References

1. Adeola, H. A., Smith, M., Kaestner, L., Blackburn, J. M., & Zerbini, L. F. (2016). Novel potential serological prostate cancer biomarkers using CT100+ cancer antigen microarray platform in a multi-cultural South African cohort. Oncotarget, 7(12), 13945.
2. Suwarnalata, G., Tan, A. H., Isa, H., Gudimella, R., Anwar, A., Loke, M. F., & Vadivelu, J. (2016). Augmentation of Autoantibodies by Helicobacter pylori in Parkinson's Disease Patients May Be Linked to Greater Severity. PloS one, 11(4), e0153725.
3. Liew, J., Amir, A., Chen, Y., Fong, M. Y., Razali, R., & Lau, Y. L. (2015). Autoantibody profile of patients infected with knowlesi malaria. Clin Chim Acta. 448, 33-38.

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