Identification of Novel Ovarian Cancer Biomarkers by Profiling the Autoantibody Repertoire

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Summary

In this study, serum samples were obtained from Discovary Consortium (Trinity College Dublin) and were screened against high density protein arrays to identify cohort specific autoantibody profiles to identify potential candidate biomarkers for diagnosis of early stage ovarian cancer.

1. Introduction

A lack of specific disease symptoms means only 20% of ovarian cancers are diagnosed at an early stage (Stage 1). There are currently no reliable biomarkers for the diagnosis of early ovarian cancer[1] highlighting the urgent need for diagnostic biomarkers. Profiling of the circulating antibody repertoire in human serum with protein expression libraries has assisted in the identification of autoantibodies associated with neoplastic events in a wide variety of human cancers. Autoantibodies to cancer antigens can be detected up to 5 years before a tumour can be identified by other means[2], meaning autoantibodies are an extremely attractive biomarker entity as they are present in blood and easily adapted into current diagnostic platforms.

We have performed autoantibody identification screening using a high content human protein expression library on serum samples from a well characterised patient cohort with stage III serous papillary adenocarcinoma. We have also performed this screening on a cohort of stage I ovarian cancer of mixed pathologies and on a cohort of patients with benign ovarian pathologies.

2. Aims and Objectives

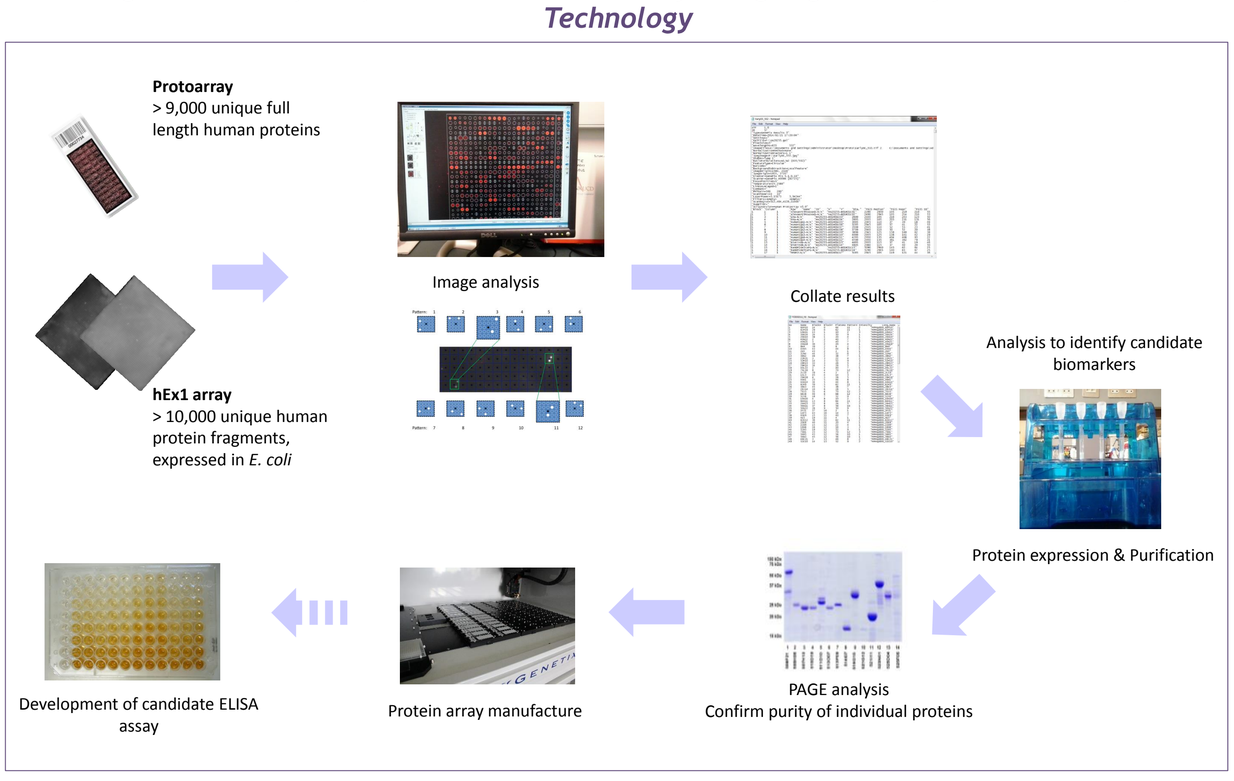
The objectives of this research were:

- Use high density protein arrays to identify the autoantibody profile of ovarian cancer, benign ovarian disease and healthy control serum samples
- To identify candidate biomarkers for the diagnosis of early ovarian cancer
- To express and purify the selected proteins for future validation studies

3. Methods

Study approval was obtained from SJH/AMNCH research ethics committee. The autoantibody response was identified using two human protein array platforms (n=>10,000 proteins); proteins expressed in E.coli (hEx1, ImaGenes)[3] and full length, modified proteins expressed in insect cells (Protoarray, Invitrogen). Using the hEx1 library, 22 early and 20 late OC serum samples, 15 benign ovarian disease samples and 26 healthy/control samples were profiled. Using protoarrays, 7 early and 5 late OC serum samples, 5 benign ovarian disease and 5 healthy control samples were profiled. Candidate protein biomarkers were expressed, purified and arrayed onto 16-pad nitrocellulose coated FAST slides using QArray technology. Microarray scanning was performed using a GenePix 4000B Axon set to scan at the ratio wavelength setting of 532nm and 635nm.

Figure 1: Workflow for Biomarker Discovery using High Density Protein Array Technology



4. Results

Novel autoantigens were identified by both array screening platforms (Figures 2 & 3). The most robust autoantibody identified to date in this study was the p53 antigen identified using the hEx1 platform[4]. Autoantibodies to p53 have been detected in 25% of late ovarian cancer samples. Approximately 250 proteins have been identified as being associated with initiating an autoantibody response in early OC (detected in early OC samples and not associated with late OC samples, healthy/control samples or with benign ovarian disease). These proteins include Serine threonine kinase involved in cell adhesion and migration; Proteins associated with endocytic and exocytic machinery; Component of the COP9 signalosome complex; Transcriptional repressor; Inhibitory subunit of a nuclear protein phosphatase.

Figure 2: Image of hEx1 array taken with a CCD camera after serum screening and array processing. Clearly evident are the positive signals that indicate an AAb binding event. The black dots are ink guide dots are used for orientation during image analysis

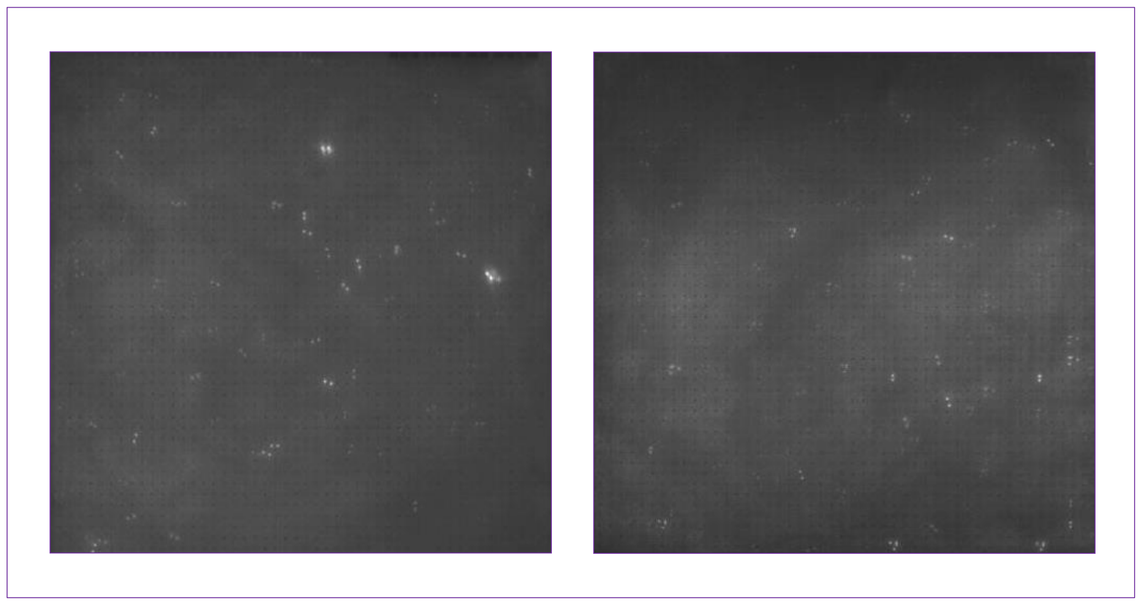
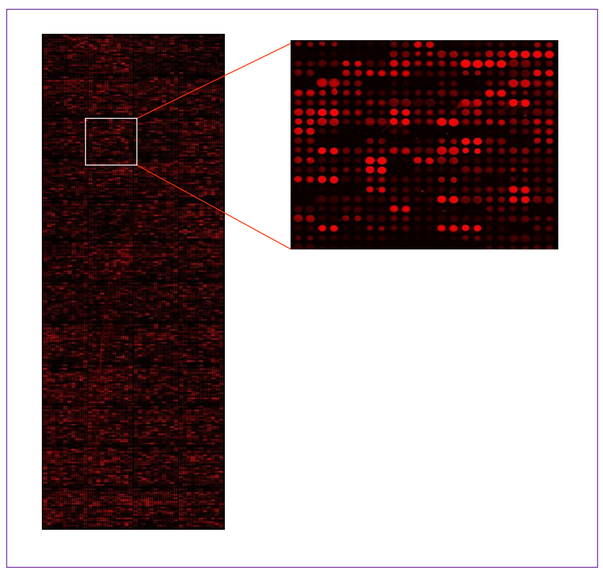
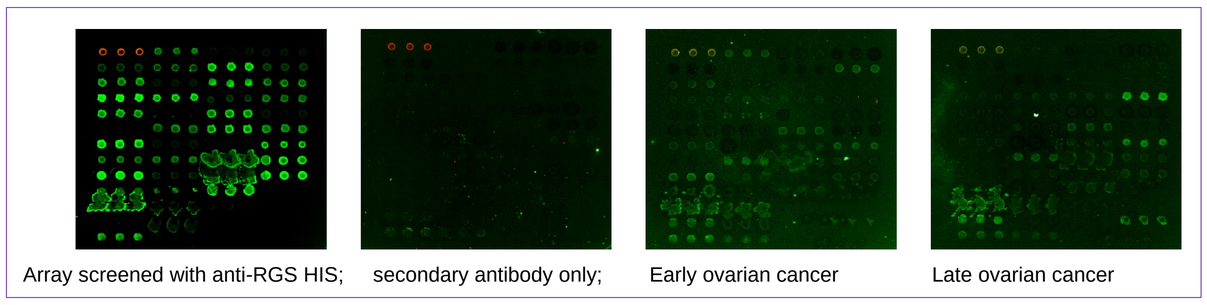


Figure 3: Image of Protoarray after serum screening and array processing, array scanned using a GenePix 4000B Axon scanner at wavelength 635nm



Forty of the proteins identified as initiating an autoantibody response were selected for initial optimisation and were arrayed in triplicate on nitrocellulose coated slides, with 16 printing pads per slide. This enabled the screening of 15 serum samples per slide, and one control screening to assess array quality from the print run. Proteins identified by early ovarian cancer specifically were selected, in addition to proteins not identified by early ovarian cancer but in other samples screened and also proteins bound by the natural autoantibody profile.

Figure 4: Images of four arrays after screening. The protein used were HIS-tagged enabling the detection of proteins using an antibody to HIS.



5. Discussion

Protein array platforms can be used to identify autoantibody profiles in OC serum. Analysis of these profiles enables the identification of candidate biomarkers for diagnosis of OC. Further optimisation is required for the validation of the candidate biomarkers however with the initial cohort of proteins selected for optimisation, differences in autoantibody expression have been observed between the different disease classifications.

References

1. Deligdisch L. Early Diagnosis of Ovarian Cancer. Is It Possible ? Med J Obstet Gynecol. 2013;1(1):1003–4.
2. Tan HT, Low J, Lim SG, Chung MCM. Serum autoantibodies as biomarkers for early cancer detection. FEBS J. 2009;276(23):6880–904.
3. O'Kane SL, O'Brien JK, Cahill DJ. Optimized autoantibody profiling on protein arrays. Methods Mol Biol. 2011;785:331–341.
4. Murphy MA, O'Connel DJ, O'Kane SL, O'Brien JK, O'Toole S, Martin C, Shiels O, O'Leary JJ, Cahill DJ. **Epitope presentation is an important determinant of the utility of antigens identified from protein arrays in the development of autoantibody diagnostic assays.** J Proteomics. 2012 Aug 3;75(15):

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