Identification of Novel Ovarian Cancer Biomarkers by Profiling the Autoantibody Repertoire

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Summary

In this study, serum samples were obtained from Discovery 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 cancer1 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 means2, 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/ANWH 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)1 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 protoaarays, 7 early and 5 late OC serum samples, 5 benign ovarian disease and 5 healthy control samples were profiled. Candidate 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 523nm 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 platform1. 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 signalsome complex; Transcriptional repressor; Inhibitory subunit of a nuclear protein phosphatase.

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