The Role of Pathway Analysis in Biomarker Selection for Ovarian Cancer

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Summary

In this study, pathway analysis was used to investigate antigens identified as binding to autoantibodies from the serum from ovarian cancer patients and relevant controls.

1. Introduction

Currently there are no reliable biomarkers for the ovarian cancer (OC) diagnosis. A lack of specific symptoms of the disease means only 20% of ovarian cancers are diagnosed at an early curable stage (Stage 1).

Autoantibodies are an attractive biomarker entity as:

- they are present in blood and
- can be adapted into current diagnostic platforms[1]

It is accepted that the complexity of cancer means that a panel of biomarkers will be required as a diagnostic test rather than the more traditional approach of identifying a single biomarker.

Due to the large number of proteins identified as bound to autoantibodies from serum of ovarian cancer patients, we investigated the potential of using pathway analysis to aid biomarker discovery[2] using a cohort of patient samples.

2. Aims and Objectives

The objectives of this research were:

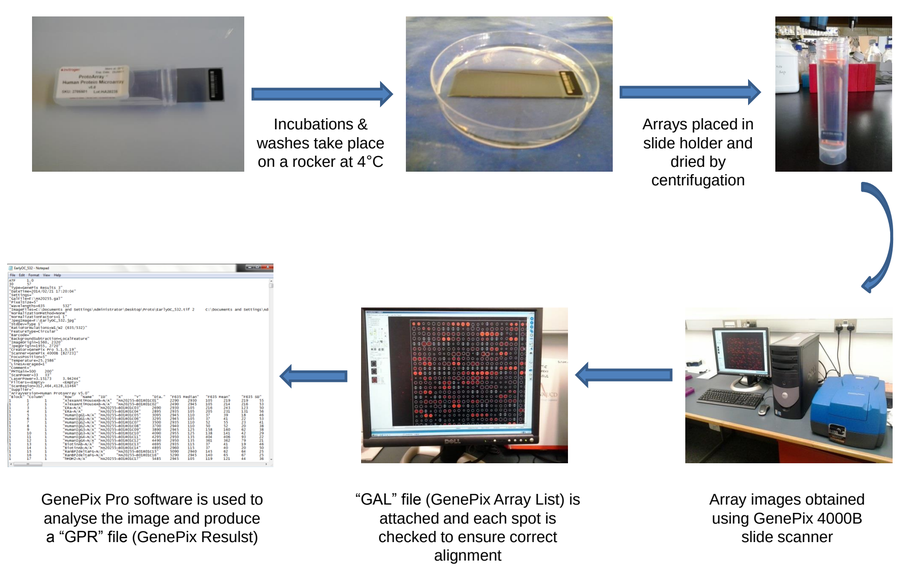
- use pathway analysis to support biomarker selection
- to gain further understanding of the mechanisms of disease pathogenesis/progression of ovarian cancer.

3. Methods

Autoantibodies associated with ovarian cancer were identified and pathway analysis was performed to determine if autoantibodies can be indicative of pathway dysregulation associated with malignancy

Serum was obtained from the Discovary Bioresource (Trinity College, Dublin). Twenty histology specific serum samples were screened on Invitrogen Protoarrays: 5 Normal; 5 Benign (Serous cystadenoma); 5 Stage 1 OC (Serous papillary adenocarcinoma); 5 Stage 3/4 OC (Serous papillary adenocarcinoma).

Figure 1: Workflow of Autoantibody Profiling using High Density Protein Arrays



Pathway analysis of identified antigens were analysed using InnateDb. Autoantibody (AAb) responses were identified as cohort associated if they were present in 40% (2/5) or above of cohort serum, and present in 20% (1/5) or less of serum from other cohorts.

Analysis was then performed on antigens identified on larger cohorts of samples (identified using an alternative high density protein array platform). Samples screened: 15 early OC (mixed histology), 10 benign (mixed histology), 20 Stage 3/4 OC (serous papillary adenocarcinoma) and 15 healthy controls.

4. Results

Autoantibodies to p53 in these serum samples were confirmed by ELISA in 20% of late stage OC patients with 100% cancer specificity which is in line with published data.

Proteins of interest identified as binding to autoantibodies include:

- Serine threonine kinase involved in cell adhesion & migration
- 2 proteins associated with endocytic or exocytic machinery,
- Component of the COP9 signalosome complex,
- Transcriptional repressor
- Inhibitory subunit of a nuclear protein phosphatase

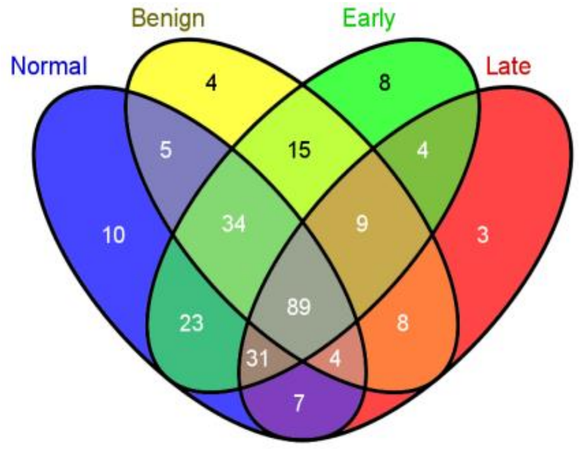
Table 1: Pathways identified in all serum samples screened.

	Benign	Early OC	Late OC	Healthy
Metabolism of proteins		20%	15%	41%
Gene Expression	20%	20%	20%	35%
3'-UTR-mediated translational regulation		20%	15%	35%
Influenza Infection		20%	15%	29%
Signalling by EGFR		13%	10%	6%
Apoptosis		13%	15%	6%
Signalling by Wnt	10%	13%	10%	6%
Signalling by NGF		7%	5%	

Table 1 details pathways identified as being associated with OC. A total of 51 pathways have been identified as Ovarian associated (not identified in Normal, see Figure 2). Eight of these pathways were identified as being associated with early ovarian cancer including:

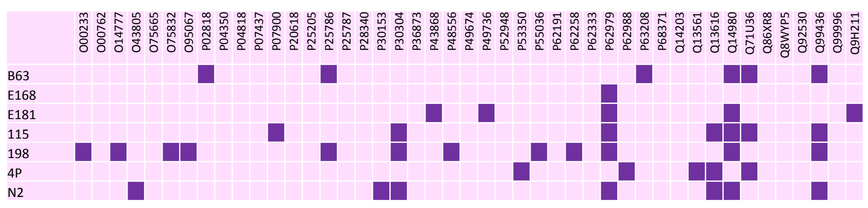
- Regulation of Eukaryotic Initiation Factor-2 (eif2)
- p53 pathway

Figure 2: Overview of the number of pathways identified in each cohort of serum samples, indicating the number of pathways identified as associated with a single cohort of samples



Some potentially significant pathways were identified in OC. For example, 45 proteins of a total of 304 proteins present in the "REACT_152: Cell Cycle, Mitotic" pathway are represented on the protein arrays used. The pattern of detection of these 45 proteins in OC serum is shown in Figure 3. These data indicate that serum autoantibodies have the potential to identify potentially important pathways and with more extensive screening data it may be possible to use this to a) highlight proteins for inclusion on a candidate protein panel for validation and b) obtain insight into OC pathogenesis.

Figure 3: Overview of the proteins detected in the Cell Cycle, Mitotic pathway by screening on high density protein arrays.



5. Conclusions

- Autoantibody profiling of serum samples identifies large numbers of autoantibodies which may be involved in disease formation and/or progression.
- Pathway Analysis can be used to mine large datasets for individual pathways that could be indicators of mechanisms of disease.
- The utility of interrogating signalling pathways identified by autoantibody profiling has yet to be determined.

References

1. Murphy MA, O'Connell DJ, O'Kane SL, et al. 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;75(15):4668-4675.

2. Ummanni R, Mannsperger H, Sonntag J, et al. Evaluation of reverse phase protein array (RPPA)-based pathway-activation profiling in 84 non-small cell lung cancer (NSCLC) cell lines as platform for cancer proteomics and biomarker discovery. *Biochim Biophys Acta*. 2013. 1844(5):950-959.

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