

Screening and Profiling Protein Expression in Human Cancer Serum using Antibody Array Technologies



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Abstract

There is a growing need for technologies that enable discovery and validation of protein biomarkers in human serum/plasma. Antibody microarrays have been used successfully to rapidly identify and characterize protein expression in a targeted approach. In this study, antibody arrays were used to interrogate proteome differences in whole serum and in serum that had been depleted of twenty high abundance proteins. The depletion technology enhanced the identification of the lower abundance tissue leakage proteins, as compared to non-depleted serum samples. Antibody arrays were also used to profile differential protein expression between serum from normal and diseased patients. Proteins were identified which displayed significantly different expression levels between the samples. Results were validated with ELISA analysis. This study showed that antibody arrays are a powerful tool for rapid expression profiling of proteins and may potentially be applied to biomarker discovery and validation in diseased serum samples.

Introduction

Antibody arrays have been a promising and inexpensive tool for bulk analysis of protein level changes in human plasma and serum. These analyses have lead to proteomic profiling of a number of disease states, as well as biomarker discovery. Here, we show the value of using a series of Panorama® antibody microarrays comparing normal and cancer serum samples to identify potential disease biomarkers. The arrays chosen for this work contained antibodies for proteins with known significance in intracellular processes. These arrays were used because it is believed that biomarkers exist in the low abundance tissue leakage proteins that make up approximately one-third to one-half of the thousands of proteins found in blood. In our study, we have also highlighted the contribution that depletion gives to the discovery/validation of the low abundance tissue leakage proteins on the antibody microarrays.

Materials and Methods

Serum Samples

Serum samples were obtained through Genomics Collaborative. Cancer samples were from either hepatocellular carcinoma (36-year old Vietnamese male) or renal cell carcinoma (66-year old Caucasian male) patients. Normal samples were from a 51-year old Caucasian male.

Serum Depletion

Depletion was completed using the ProteoPrep® 20 Immunoaffinity Depletion Kit (PROT20) and following the supplied protocol.

ELISA Analysis

Serum samples were coated onto 96-well ELISA plates. Plates were incubated with purified primary antibodies corresponding to those on the arrays. Plates were washed and incubated with HRP-conjugated secondary antibodies. After a final wash, the plates were visualized using TMB substrate, and stopped with 1 M HCl. The absorbance was measured at 450 nm.

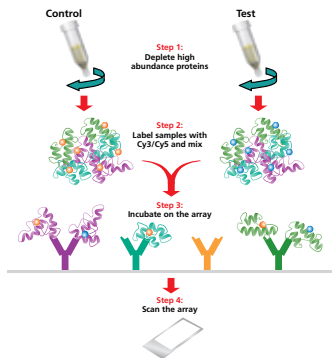


Figure 1: Microarray Analysis Workflow

Serum samples were first depleted of twenty of the high abundance proteins. The depleted samples were then labeled with either Cy3 or Cy5 (Amersham) and mixed at equal protein amounts to allow for parallel analysis. The labeled serum samples were incubated on both the Panorama Antibody Array – p53 Pathways (PPAA4) and the Panorama Antibody Array – Cell Signaling (CSAA1) for 30 minutes. Following incubation, the slides were scanned using a ScanArray Express (Perkin Elmer) and analyzed using ImaGene 7.0 software (BioDiscovery).

Results

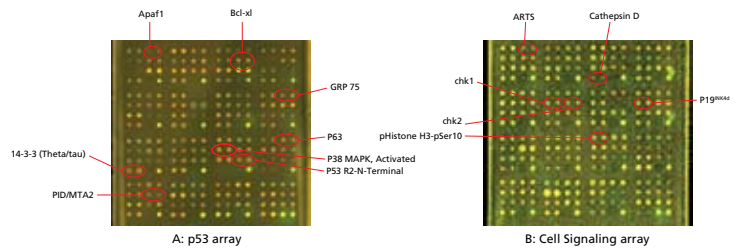


Figure 2: Benefits of Depletion by Comparison of Whole and Depleted Normal Human Sera Using Panorama p53 (A) and Cell Signaling (B) Arrays

Each Panorama p53 array was incubated with 100 µg of both depleted and whole (non-depleted) serum conjugated with either Cy3 or Cy5. A dye swap was performed to confirm results. In the comparisons, the whole serum is green and the depleted serum is red. Select proteins are identified. Note that only the top half of the Cell Signaling array slide is shown. As seen in the comparisons above, more proteins are visible in the depleted serum than in the whole serum. The depletion has made the less abundant proteins visible.

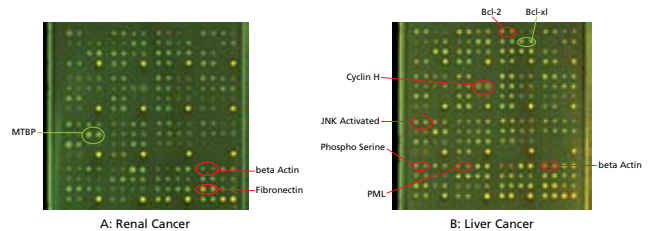


Figure 3: Differential Expression Levels of Various Tissue Leakage Proteins in Cancer Serum Samples Using Panorama p53 Array

Following depletion, each Panorama p53 array was simultaneously incubated with a mixture containing equal amounts of normal and cancer serums (Renal cancer for slide A, Liver cancer for slide B) conjugated with either Cy3 or Cy5. A dye swap was performed to validate results. As seen in the slides, a number of spots were differentially expressed with the cancer samples when compared to the normal control. In both comparisons above, the normal serums are labeled red and the cancer serums are labeled green. Therefore, a red spot would indicate down-regulation in the cancer sample, and a green spot would indicate up-regulation in the cancer sample. Select proteins are identified.

Similar results were seen using the Cell Signaling array. Additional proteins found to be significantly different using the Cell Signaling array include, but are not limited to: alpha Catenin (up-regulated in both cancer samples), MAP Kinase (Erk1 + Erk2) (up-regulated in both cancer samples), Calmodulin (up-regulated in the liver cancer sample), Cyclin D1 (down-regulated in the liver cancer sample), DOPA Decarboxylase (down-regulated in both cancer samples), and Synculein (down-regulated in the renal cancer sample). Data not shown.

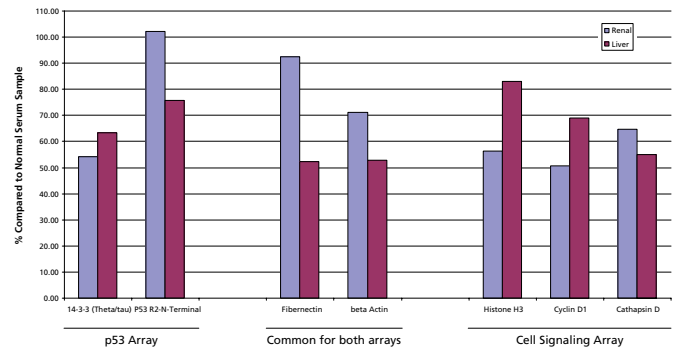


Figure 4: Validation of Results by ELISA Analysis

Changes in the expression of various tissue leakage proteins between the cancer and normal serum samples were validated by ELISA analysis. The proteins analyzed were significantly different in the depleted vs. non-depleted and/or in the cancer vs. normal samples. An example of the results is illustrated above.

Conclusions

- Depletion allows for greater visibility of otherwise difficult to detect proteins on the antibody arrays.
- Multiplexed array assays (using two dyes on the same array) allow for a quick, direct, and inexpensive comparison of normal and diseased serum samples.
- As expected with the number of tissue leakage proteins present in blood serum, microarrays containing antibodies important to intracellular pathways are useful in the detection of potential biomarkers.
- This work shows proof-of-principle that the Panorama antibody microarrays can enable disease-state protein profiling.
- Due to the variability between individuals, future work would include the analysis of a large cancer sample population to determine protein profiles and validate biomarkers for a select type of cancer.

References

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