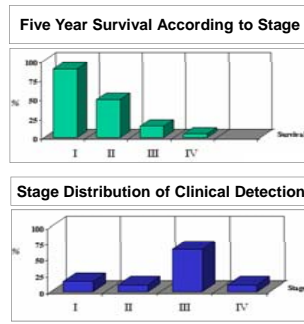


IDENTIFICATION OF A FOUR MARKER PANEL FROM THE SERUM FRAGMENTOME FOR THE CLASSIFICATION OF STAGE I OVARIAN CANCER

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INTRODUCTION

All cancer patients benefit from early detection. The need for development of early screening methods for epithelial ovarian carcinoma is particularly urgent. Ovarian cancer is the fourth leading cause of cancer related deaths among women in the United States. Regrettably, 70-75% of new diagnoses will be Stage III or IV with a predicted 5-year survival of ca.15%. This survival rate has remained essentially unchanged over the past 40 years despite the advent of intensive radical surgical approaches and new therapeutic interventions. In contrast, if cancer is detected when confined to the ovary (Stage I), the 5-year survival approaches 90%, requires less radical operations, and may not require adjuvant chemotherapy. More sensitive and specific tests for earlier detection would greatly improve patient survival rates by facilitating early treatments such as surgical intervention.



METHODS

The ovarian cancer serum biomarker study was performed using the PerkinElmer BioXPRESSION Platform (below).

Sample Preparation

1. Adhere capture of cancer proteins allowing non-competitive detection.
2. Dilute Protein & Protein Fragment Mixture.
3. Concentrate & Clean up (20µm) (Magnetic).
4. Digest Eluate with HCTP MALDI (On Target).

High-Throughput Biomarker Screening

MALDI O-TOF MS

- Mass accuracy: <10 ppm external calibration
- Mass stability: 10 ppm over several hours using external calibration
- Resolution: 15,000 (FWHM)
- Detection Limit: Sub-femtomole
- Throughput: >10,000 samples/day

Biomarker Detection

PROGENESIS PG600 Software

- Robust Biomarker Discrimination Analysis Software from Nonlinear Dynamics
- Intelligent Peak Detection
- Spectral Display Options
- Atlas Differential Display
- APCGMA & NJ Clustering
- Grouping & Averaging According to Class

Biomarker Identification

Accurate mass, isotopically-resolved, differentially-expressed BioXPRESSION processed peptides were selected for LC-MS/MS

Export accurate identification services via a part of the BioXPRESSION Platform (ProteinScribe)

Study & Clinical Sample Information:

The ovarian study set consisted of 560 total samples. The number of serial samples collected from individual patients varied from 3 to 22 samples per patient. Samples from cancer patients were also assayed for CA125 levels. There were 213 samples with low (below 22 ng/ml) and 239 samples with high (above 22ng/ml) CA 125.

Healthy control individual serum samples were collected from unaffected women (no serial samples), ovarian cancer samples were procured in a gynecological oncology clinic from symptomatic women who were later surgically staged and found to have epithelial ovarian carcinoma. Each sample was accompanied by a verified pathology diagnosis. Specimens were collected in red-top Vacutainer Tubes and allowed to clot for 1 h on ice, followed by centrifugation at 4 °C for 10 min at 3000 rpm. The serum supernatant was divided in aliquots and stored at -80 °C until needed. Classification model construction was constructed by Martin O'Gorman (Nonlinear Dynamics, UK). Models, created from a training set of 114 disease and control spectra (out of a total of 1154 spectra) were then applied to the testing set (remainder of samples).

Treatment	Number of samples	Analysis replicates
Healthy (control)	168	324
Early stages of ovarian cancer (low CA125)	213	639
Late stages of ovarian cancer (high CA125)	239	717
Total samples	560	1680

Ovarian cancer serum samples were obtained courtesy of E. Petricoin, FDA, G. Whiteley, NCI and D. Fishman, NYU.

RESULTS

The BioXPRESSION Platform (PerkinElmer SCIEX) provides high-resolution, high-sensitivity analysis of the serum fragmentome. High throughput screening of carrier-protein bound protein fragments by MALDI O-TOF MS (ProTOF 2000, PerkinElmer SCIEX) rapidly identified differentially-expressed proteins and peptides (Figure 1).

Scale-up of the BioXPRESSION Platform resulted in accurate sequence information by LC/MS/MS (Table 2). Multiple protein fragments (with very high identification probabilities) were obtained for most serum proteins (Table 3).

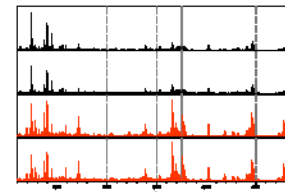
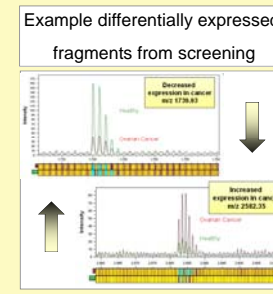


Figure 1. ProXPRESSION Biomarker HT Kit processed sera replicates analyzed by high resolution MALDI Orthogonal-TOF MS (ProTOF 2000, PerkinElmer SCIEX). Mass range displayed = 1600 – 4300 Da. Top (black) = Control, Bottom (Red) = ovarian cancer.

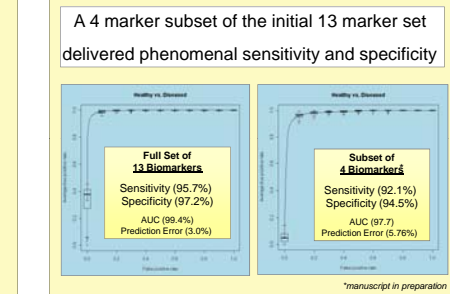
Protein	Observed Fragment	MW (Da)	P (Pep)	Protein	Observed Fragment	MW (Da)	P (Pep)
Transferrin	R.VTTHALLSPYEVYTVV	2932.34	2.98E-04	Transferrin	S.SVDFPQSTKTEGR	2113.58	3.95E-04
Transferrin	R.VTTHALLSPYEVYTVV	2932.34	2.98E-04	Transferrin	S.SVDFPQSTKTEGR	2113.58	3.95E-04
Transferrin	R.VTTHALLSPYEVYTVV	2932.34	2.98E-04	Transferrin	S.SVDFPQSTKTEGR	2113.58	3.95E-04
Transferrin	R.VTTHALLSPYEVYTVV	2932.34	2.98E-04	Transferrin	S.SVDFPQSTKTEGR	2113.58	3.95E-04
Transferrin	R.VTTHALLSPYEVYTVV	2932.34	2.98E-04	Transferrin	S.SVDFPQSTKTEGR	2113.58	3.95E-04

Table 2. Partial listing of some of the protein fragments identified using the BioXPRESSION Platform (PerkinElmer SCIEX). Thousands of fragments representing over 200 human serum fragments have been identified.



Sequence information for some of the key peptide fragments

m/z	Full expression error in healthy	P Value	Sequence ID
1691.54	1.1E-06	0.000000	S.VTTHKRVESKLLR
1719.99	2.48	0.000000	S.VTTHKRVESKLLR
1719.99	1.83	0.000000	S.VTTHKRVESKLLR
1719.99	1.36	0.000000	S.VTTHKRVESKLLR
1861.01	-1.56	0.000000	S.VTTHKRVESKLLR
2021.11	-1.47	0.000000	S.VTTHKRVESKLLR
2091.54	2.09	0.000000	S.VTTHKRVESKLLR
2017.01	2.30	0.000000	S.VTTHKRVESKLLR



CONCLUSIONS

The BioXPRESSION Platform is a powerful biomarker screening platform. Many differentially-expressed protein fragments were identified from human serum. A panel of four protein fragment biomarkers classified early stage disease from non-disease with very high sensitivity and specificity. While many of the more differentially-expressed biomarker candidates represent fragments of coagulation proteins, many interesting fragments (PPARs, Notch4, angiogenesis inhibitors, etc.) were also identified. Biomarker subsets have been submitted for publication.