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Proteomic Based Analysis of Ovarian Cancer Pathways

Abstract

Recent genomic-based studies have suggested ovarian cancer is a disease of copy instability, with 63 regions of focal amplification, rather than a disease driven by mutation. However, this analysis has been unable to identify changes in protein expression, modification, and activity. In this study we developed a highly quantitative MS/MS approach to examine the ratio of protein levels, combining TMT labeling with a number of motif and site-specific antibodies to identify signaling pathways differing between tumor and normal specimens. We identified over 1400 uniquely upregulated proteins, and over 1600 upregulated posttranslational modification sites across 79 tumors and 19 normal tissue samples. We were able to survey activity of over 400 kinases and over 100 other enzymes as well as the substrates associated with them. Our study was able to map these upregulated proteins based on abundance and activity level to their regions of focal amplifications. By focusing on activated cell circuitry, the approach outlined here was able to provide insight into cancer biology not available at the chromosomal and transcriptional levels. This approach may reveal new connections among pathways and candidates for therapeutics and diagnostics.

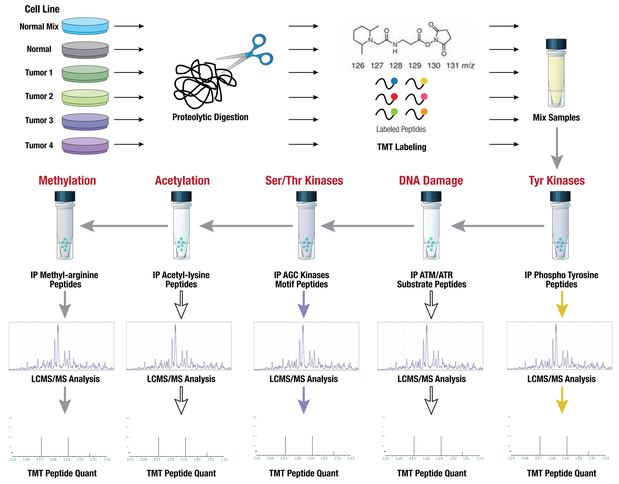


Figure 1. 2mg of peptides from trypsin digested tissue were labelled with TMT reagent and combined into 20 six-plexes. The mix of normals was a concatanation of the normal samples. Following a test mix to ensure a 1:1:1:1:1:1 mix across all channels, samples were appropriately combined and serially fractionated through immunoprecipitation with antibodies specific for phosphotyrosine, ATM/ATR substrate motif, AGC/CAMK/STE family kinase motif, acetyl-lysine, and methyl-arginine. For all phospho-spaces, an additional Immobilized Metal Affinity Chromatography (IMAC) step was used to further enrich samples. The peptides from each immunoprecipitation were analyzed by LCMS/MS using a Q-Exactive® LC-MS/MS, with quantification of the TMT labels enabled by CID fragmentation. Data processing was performed using CORE. (Protein Sieve, Protein Assembler, Protein Quant.) In the analysis of our data, we defined upregulated protein meets the criteria of having >5 fold change based on 2 sigma cutoff (95% confidence).

Sequential IP Reagents

Ab Target	Clone #	Motif
pY-1000	D1G10/D2D1	Phospho Tyr
AGC/PSD Akt substrate AMPK ATM/ATR substrate ATM/ATR substrate	D3E5/D8D9/D4E2/D8B11 100B7 D72H3/D78G9 D23/D69 D14/D86	AGC/CAMK/STE kinase acitivation loop RXX(s/t) LXRXXT(S) (s/t)Q (s/t)Q
Ac-K K-Me R-Me Epigenetic Regulator Mix	D10G3/11D/16E D4P3J, D3Z9J, D8R1C Me-R4-100 -	Acetyl lysine K-Me R-Me -
Ubiquitin Library	D4A7A10	kGG

Table 1. Motif antibodies used with corresponding targets.

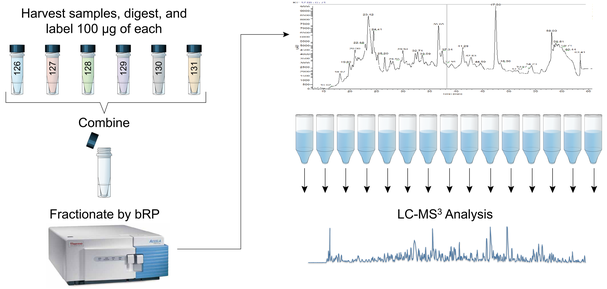


Figure 2. Samples were purified over Sep-Pak® cartridge C18 columns before being subjected to peptide quantification. 100ugs of each sample was then labeled with the appropriate TMT reagent according to the optimized protocol, combined at a 1:1:1:1:1:1 ratio, and again purified over a Sep-Pak® cartridge C18 column. Samples were then fractionated over a ACCELA HPLC using a 4.6X150mm ZORBAX® 300 Extend-C18 (5um particle size with 300 A-pore). 96 fractions were collected over the 60-minute gradient. The fractions were then combined to give a total of 12 and these fractions were again purified over a C18 micro column. Each purified fraction was then subjected to MS/MS/MS analysis.

Integrattion of Proteomic Data

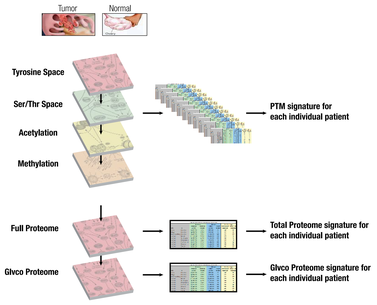
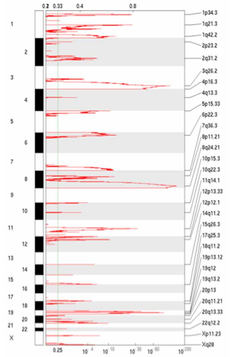


Figure 3. Every patient and normal sample was profiled through Tyrosine, Ser/Thr, Acetylation, and Methylation Spaces. This PTM signature was combined with Total Proteome and Glycoproteome analysis to assemble a unique proteomic signature for each patient.

Overlap Between Genomics and Proteomics



Chromosome	Overlap TCGA and Proteomics	Identified by Proteomics in the surrounding region
chr1	27	107
chr2	25	12
chr3	12	50
chr4	9	0
chr5	18	18
chr6	42	20
chr7	29	10
chr8	27	27
chr10	9	8
chr11	4	15
chr12	23	36
chr14	12	12
chr15	9	2
chr17	26	16
chr18	7	3
chr19	31	101
chr20	15	12
chr22	4	13

From proteomic data

The Cancer Genome Atlas Research Network. (2011) Nature 478, 609-615.

Figure 4. In ovarian cancer, 63 regions of focal amplification that have been identified (left side.) Our proteomics approach mapped many upregulated proteins directly to or close to these regions of amplification (right side).

Proteins Identified by Proteomics in Ovarian Cancer

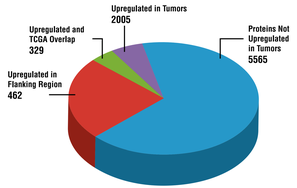


Figure 5. A breakdown of proteins identified by proteomics based on tumor to normal ratio, and overlap with TCGA data in ovarian cancer.

Deregulated Pathways in Ovarian Serous Cancer Identified by Proteomics

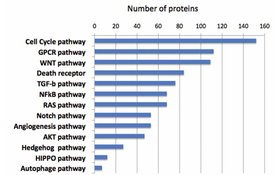


Figure 6. Gene enrichment analysis identified a number of deregulated pathways in ovarian cancer.

Survey of Deregulated Enzymes

Targets	Identified	High in T vs N
Kinases	403	207
Acetylases or Deacetylases and proteins in complex with them	115	55
Methylases or demethylases and proteins in complex with them	116	64

Table 2. Number of enzymes identified in ovarian cancer samples.

Molecular Mechanisms of Ovarian Cancer

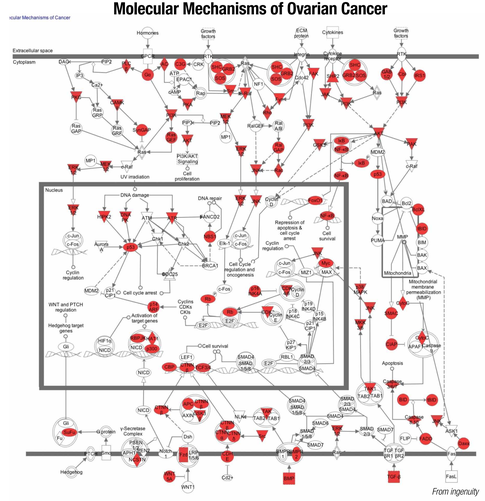


Figure 7. Analytic tools from Ingenuity Systems, Inc. were used to map upregulated proteins in tumors (red) to established cancer pathways.

Summary

- We have developed a unique proteomic approach to survey signaling pathways in cancer, revealing information not available at the levels of chromosomal or transcriptional analysis.
- This approach was applied to ovarian cancer, identifying new deregulated pathways and possible drug targets.

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