Glycan Biomarkers of Ovarian Cancer by LC-MS/MS: Comparing *N*-Glycans Released From Proteins in Cell Lines and Tissues

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INTRODUCTION

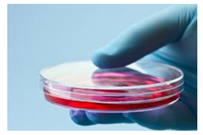
Epithelial ovarian cancer is the sixth most common cause of cancer worldwide with the highest mortality rate within gynecological cancers. Most patients are often diagnosed when the disease has already metastasized to distant sites, resulting in a poor 5 year survival rate of 15-30% when diagnosed at late stages III and IV as compared to 80-95% when diagnosed at early stages I and II[1].

Post-translational modifications, such as glycosylation, have been shown to be associated with cancer. Aberrant glycosylation is the result of alterations in glycosylation enzymes which lead to altered glycan structures. Since a majority (90%) of ovarian cancers are epithelial in origin[2], cell surface *N*-glycans display unique epitopes upon malignant transformation and have the potential to be exploited as glycan biomarkers. Besides that, these surface *N*-glycans can also be utilized for the development of therapeutic drugs which target specific glycosyltransferases involved in the cellular glycosylation pathway[3].

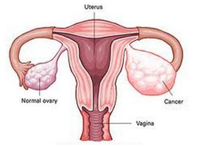
AIM

- Characterize and quantify cell surface *N*-glycosylation of normal ovarian surface epithelial cells, ovarian cancer cells and serous ovarian cancer tissue.
- Identify unique *N*-glycan structures that are present in both ovarian cancer cells and tissue based on the separation using negative ion mode PGC-LC-ESI-MS/MS fragmentation patterns, diagnostic ions and retention times.

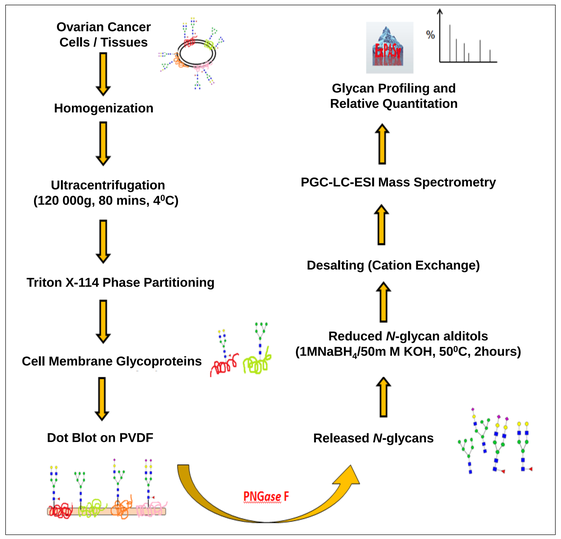
MATERIALS AND METHODS



Cell Lines:
a) Normal ovarian surface epithelial cells (HOSE 6.3, HOSE 17.1)
b) Ovarian cancer cells (SKOV3, IGROV 1, A2780, OVCAR 3)



Serous Ovarian Cancer Tissue:
a) S450
b) S520
c) S565



Statistical Analysis:
a) One-way ANOVA
b) Principal Component Analysis

MS Conditions:
a) 5 µm Hypercarb, 180 µm I.D.× 100 mm)
b) 85 min gradient of 0-45% CH_3CN in 10 mM NH_4HCO_3.
c) Flow rate: 2 µL/min
d) HPLC system (Agilent 1100) connected directly to an ESI source (Agilent 6330).
e) Scan range: m/z 200-m/z 2200.

Verification Experiments:
a) Sialidase (Enzyme) Treatment
b) Gene Expression Analysis of specific glycosyltransferase enzymes using RT-PCR

RESULTS

A) Glycosylation MS profiles reveal differences between ovarian cancer cell lines and tissues

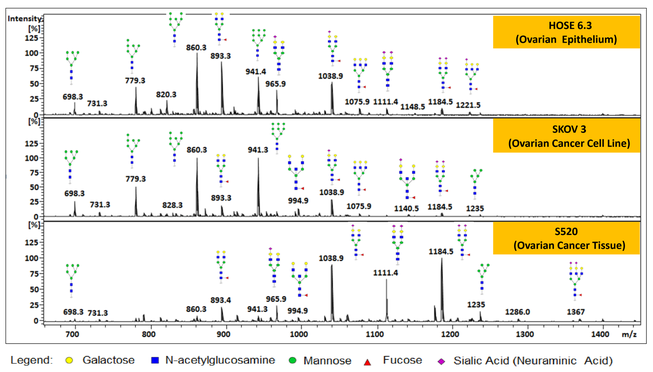


Figure 1: Representative average MS profile displaying *N*-glycan structures that are present in a) HOSE 6.3 (normal ovarian surface epithelium), b) SKOV3 (ovarian cancer cell line) and c) S520 (ovarian cancer tissue).

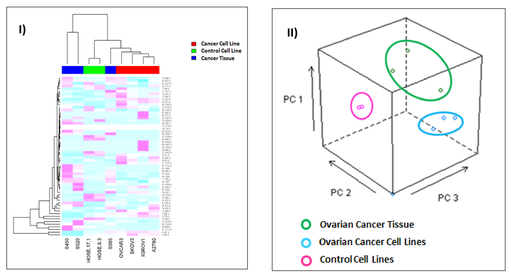


Figure 2: I) Unsupervised hierarchical clustering heatmap of 60 *N*-glycan relative intensities show clustering between tissue and cell lines. The colorgram indicates the intensity of abundance, with blue representing low, purple indicating high and white as intermediate expression. II) The samples (n=9) were projected on the first three principal components and the three main sample clusters are highlighted in colour.

- High mannose *N*-glycans represent the major structures in both the normal ovarian epithelial and ovarian cancer cell lines.
- The complex sialylated *N*-glycans are significantly up-regulated ($p<0.001$) in ovarian cancer tissue as compared to the normal ovarian surface epithelium and ovarian cancer cell lines.

➢ The relative MS ion intensities of these *N*-glycans indicate a clear distinction between:
a) normal ovarian surface epithelia and ovarian cancer cell lines
b) ovarian cancer cell lines and ovarian cancer tissue

B) Identification of specific *N*-glycan epitopes found in ovarian cancer cell lines and tissues

- ***Alpha2-6 sialylation***

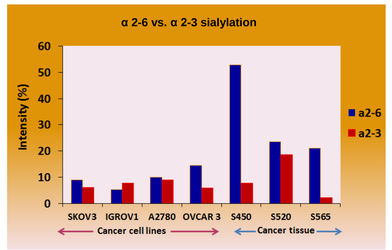


Figure 3a: Relative MS ion intensities for ovarian cancer cell lines and tissues. Data taken from nine *N*-glycan structures (hybrid and complex sialylated)

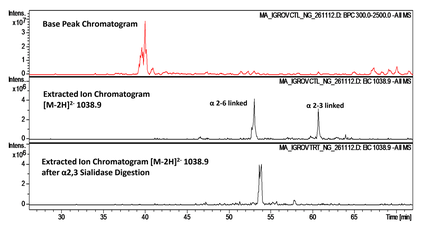


Figure 3b: Determination of alpha2-6 or alpha2-3 linked sialic acid isomers are determined based on their elution at separate retention times. The α2-6 linked sialylated *N*-glycan isomer is observed after treatment with sialidase.

- ***Bisecting N-glycans***

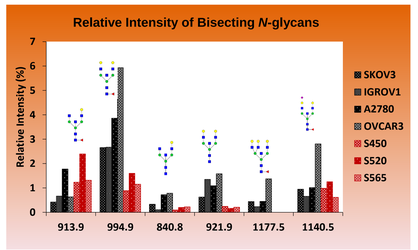


Figure 4a: Relative MS ion intensities of bisecting *N*-glycans for ovarian cancer cell lines and tissues. An example of how a bisecting-type *N*-glycan is determined is shown below (Figure 5b).

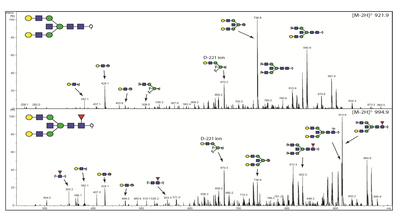


Figure 4b: MS/MS fragmentation spectra used to identify bisecting-type *N*-glycans. D-221 ion is formed when the bisecting GlcNAc attached to the innermost Man residue is cleaved from the 6-antenna comprising of Hex-HexNAc-bisecting GlcNAc-Man-Man.

- ***Verification with RT-PCR***

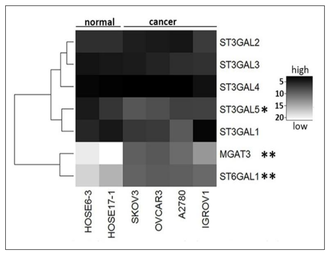


Figure 5: Gene expression analysis indicate that ST6GAL 1 (α2-6 sialylation) and MGAT3 (bisecting) are significantly upregulated in ovarian cancer cells as compared to the control normal ovarian surface epithelium.

➢ PGC-LC-ESI-MS/MS enabled the separation glycan isomers (α2,6 and α2,3-linked sialic acid).

➢ Tandem MS/MS fragmentation can provide useful fragments for the detection of bisecting-type *N*-glycans.

CONCLUSION AND FUTURE DIRECTIONS

- The data demonstrates the potential utility of mass spectrometry for the detection of cancer-specific glycan alterations as diagnostic ovarian cancer biomarkers and therapeutic anti-glycan antibody targets.
- Future work will be focused on analyzing ovarian cancer tissue samples obtained from patients (derived from the ovary, fallopian tube and peritoneum) to identify specific *N*-glycans which could facilitate their distinction.

REFERENCES AND ACKNOWLEDGEMENTS

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