Identification of Tumor-Marker Gangliosides for Early Cancer Detection: A Mass Spectrometry Approach

Rachel Culp-Hill[1], Collin Hill[1], Adele Blackler[1], William Ricketts[1]

[1]AOA Dx, Denver, CO, USA

Introduction

Tumor-marker gangliosides (TMGs) have enormous potential as diagnostic biomarkers, particularly in early stage cancer detection, despite being understudied in this context. Aberrant levels of gangliosides, including GD2 and GD3, have been identified in several cancers such as **ovarian**, breast, neural and brain, and bone cancers. AOA Dx is focused on the development of multiple platforms aimed at detecting and quantifying gangliosides in serum, with a specific focus on ovarian cancer. In line with these efforts, AOA has successfully developed a mass spectrometry platform (HPLC-MS) for the detection and quantitation of disialo-gangliosides GD2 and GD3 using a combination of targeted and untargeted techniques. Additionally, we have developed immunoassays such as TLC immunostaining and ELISA for the detection and quantitation of GD2, with further ongoing work for GD3 immunoassays. These platforms allow for the detection of gangliosides secreted into serum.

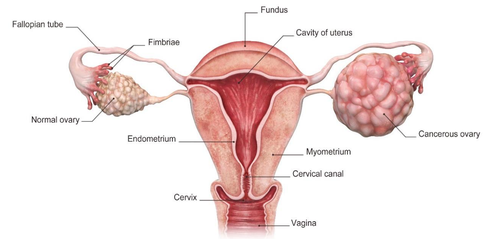
Multi-platform development

Mass Spectrometry: Untargeted and targeted ganglioside methods were developed using a qTOF and QqQ to identify and quantify GD2 and GD3 from purified GD2 standard and extracted serum samples.
GD2 Competitive ELISA: A single antibody ELISA was developed using the competitive platform. Briefly, the plate was coated with GD2 conjugated to a protein carrier, and a biotinylated antibody is mixed with extracted serum before being added to the plate. The more antibody that binds to native substrate, the less there is to bind on the plate, thus the signal is decreased in the presence of competing GD2.
GD2 TLC immunostaining: Thin layer chromatography was used to analyze extracted serum samples. The samples were spotted onto an HPTLC plate, developed with MeOH/CHCl3/CaCl2 solution, fixed in PIBM and incubated overnight with a labeled GD2 antibody. After incubation, bands were detected with chemiluminescence that were consistent with GD2.

Conclusions and next steps

All three platforms (HPLC-MS, TLC immunostaining, ELISA, and mass spectrometry) are feasible for the detection of GD2. We have also determined that our sample preparation and cleanup procedures are both robust and reproducible. Moving forward, we will begin using these platforms to analyze human samples and will continue to develop the method for analysis of GD3.

Ovarian cancer (OC) is deadly, but symptomatic

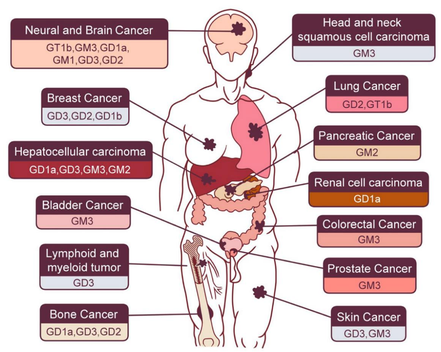


- 8th most common cancer in women worldwide[1], dubbed the "silent killer."[4]
- 94% experience symptoms, but symptoms are vague and often attributed to more common conditions[2].
- >70% cases diagnosed at stages III or IV, reducing 5-year survival rates.[3]

Survival rate could be improved by early diagnosis

- Most common path to diagnosis = transvaginal ultrasound + CA-125[5]
- Biomarkers (ex. CA-125) show low clinical sensitivity[6]
- CA-125 is **not** elevated in…
  - 50% of early-stage disease
  - 20% of advanced stage disease
- <50% diagnosed within 1 month of first doctor visit[7]
- Average time to diagnosis = 8 months

Gangliosides: powerful novel biomarkers

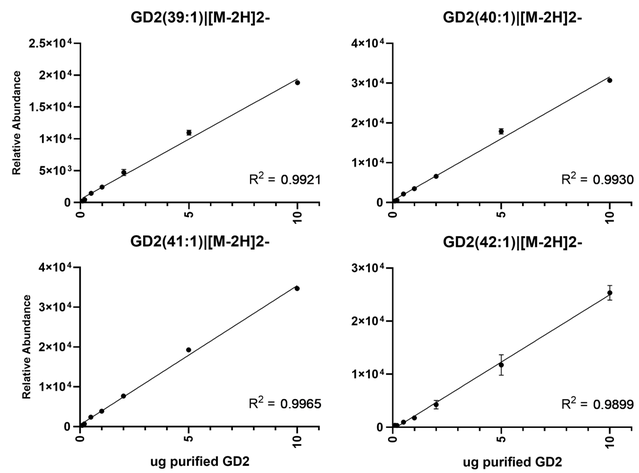


- Aberrant ganglioside levels identified in several cancers
- Heterogenous levels/distribution indicate unique disease signature
- Gangliosides relatively low in healthy serum
- Tumor marker gangliosides (TMGs) increased in cancer

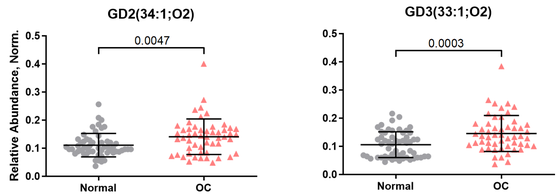
Untargeted HPLC-MS based analysis of gangliosides

Analysis was performed on purified GD2 standard (Cayman Chemical) via high pressure-liquid chromatography-mass spectrometry (HPLC-MS – Agilent 1290 UPLC and 6530 Qtof MS). Five microliters a standard curve (see below) of purified GD2 were loaded onto a Kinetex 2.6 µm C18 100 Å, LC Column (100 x 2.1 mm – Phenomenex). A 15 min gradient from 30-95% B (A = 60:40 MeOH:water + 10mM ammonium formate, B: 90:10 IPA:MeOH + 10mM ammonium formate) were used to elute gangliosides of interest. The mass spectrometer scanned in Full MS mode and performed Auto MS/MS. The mass range was between 500-2500 m/z, spray voltage was 4.2 kV, and the instrument was operated in negative mode. Metabolite assignment was performed against an in-house ganglioside library using Maven (Melamud et al. 2010)

LCMS shows good dynamic range across standard curve of purified GD2

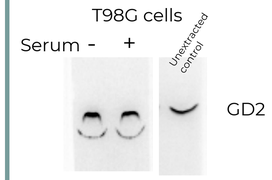


Targeted MS shows increased GD2 and GD3 in ovarian cancer

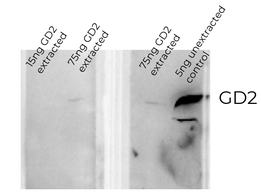


A cohort of age-matched healthy controls and ovarian cancer patient serum was extracted and run using targeted QqQ MS.

Extraction efficiency improves in physiologically relevant systems compared to GD2 + BSA



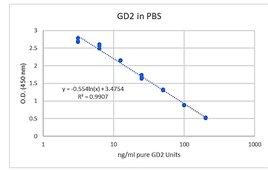
GD2 present in cells is efficiently extracted with or without serum.
TLC immunostaining results of T98G cells either spiked into serum or PBS and extracted



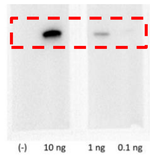
Purified GD2 with BSA added to serum is not extracted
TLC immunostaining results of purified GD2 spiked into serum and extracted

ELISA and TLC immunostaining show good dynamic range for purified GD2

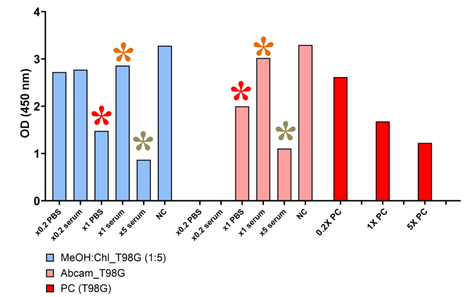
Standard curves of competition ELISA and TLC immunostaining show sensitivity down to 0.1-0.3ng purified GD2



Competition ELISA shows linear dynamic range from 200ng to 0.3ng of GD2



TLC immunostaining shows linear dynamic range from 10ng to 0.1ng of GD2



GD2 expressing cells were added to either PBS or human serum and extracted with a modified Bligh Dyer method (blue) or a lipid extraction kit (AbCam, pink.) The y-axis shows OD, so a lower signal equates to a higher level of GD2.
The 5X spike is quantitative(*), but lower amounts show lower recovery in serum (*). This phenomenon is not observed PBS (*), suggesting there is possible lipid contamination interfering with antibody binding in serum.

Adele Blackler, AOA Dx
adele.blackler@aoadx.com
www.aoadx.com