Identification of Tumor-marker Gangliosides in Serum for Early-Stage Ovarian Cancer Diagnosis

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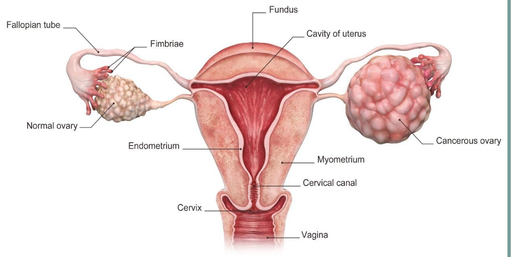
Background

Ovarian cancer (OC), the fifth leading cause of cancer-related deaths among women, is often misdiagnosed due to nonspecific symptoms and a lack of effective diagnostic tools. Consequently, late-stage detection occurs in 80% of patients when the five-year survival rate is <30%. Gangliosides are lipids involved in cell signaling and other pathways, present at low levels in healthy individuals but dysregulated in certain cancers. These tumor-marker gangliosides (TMGs) are shed into circulation from tumor cells and accessible through liquid biopsy. TMGs are emerging as promising diagnostic biomarkers for early-stage cancer detection.

Ovarian cancer (OC) is deadly, but symptomatic

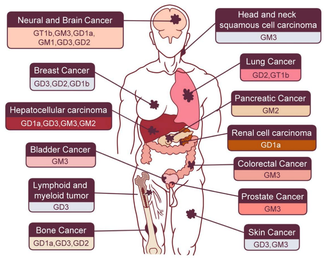


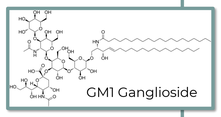
- OC is the 8th most common cancer in women[1,4]
- 94% of women experience symptoms starting at stage I, but symptoms are vague and often attributed to more common conditions[2]
- >70% of OC cases are diagnosed at stages III or IV when 5-year survival rates are <30%[3]

Survival rate could be improved by early diagnosis

- Most common path to diagnosis = transvaginal ultrasound + measurement of CA-125[5]
- CA-125 shows low clinical sensitivity and specificity[6] and is elevated in several nonmalignant conditions such as benign tumors, menstruation, peritonitis, and others.[11]
- CA-125 is also **not** elevated in 50% of early-stage OC and 20% of late-stage OC
- <50% of patients are diagnosed within 1 month of their first doctor visit[7]
- Average time to OC diagnosis is 9 months in the U.S.[8]
- Better diagnostic tools are needed to reduce time to diagnosis and detect OC in earlier stages.

Gangliosides: powerful novel biomarkers





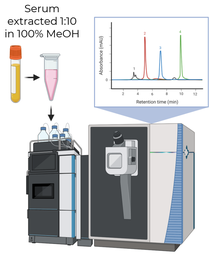
- Gangliosides are a family of sialic acid-containing glycosphingolipids. Located in the plasma membrane, they regulate many cellular processes through signaling but can be shed into circulation.
- Ganglioside levels are relatively low in healthy serum
- Aberrant ganglioside levels have been identified in several cancers[12]
- Heterogenous levels/distribution indicate potential for unique disease signatures

UHPLC-HRMS/MS as a method for ganglioside detection

We generated data using UHPLC-HRMS/MS (Vanquish + Exploris 240, Thermo Scientific) from two cohorts of serum samples.

Cohort 1	# samples
Healthy	70
Early-stage (I/II) OC	8
Late-stage (III/IV) OC	27

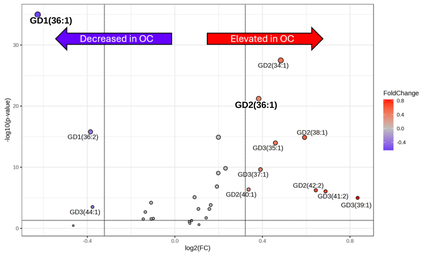
Cohort 2	# samples
Healthy	80
Early-stage (I/II) OC	77
Late-stage (III/IV) OC	137



Ganglioside Class	Species Detected
GM1	7
GM2	3
GM3	25
GD1	10
GD2	6
GD3	19
GT1	10
GT3	1
GQ1	2

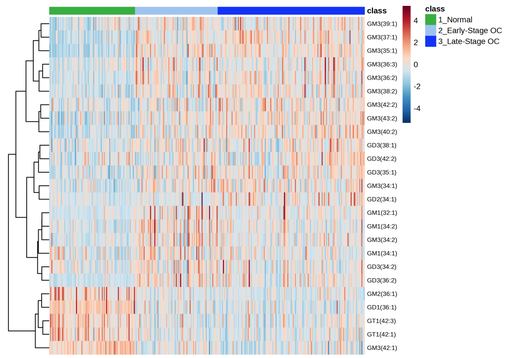
83 distinct ganglioside species were detected. Gangliosides were confirmed using ganglioside-specific fragment moieties.

Aberrant gangliosides observed in OC



Disialogangliosides were the primary focus of our first cohort. Several were altered, but we notably observed an increase in GD2(36:1) and a decrease in GD1(36:1) in OC when compared with healthy individuals.

Aberrant gangliosides observed in both early-stage and late-stage OC



In our second cohort we expanded our detection to include all ganglioside masses. We again observed clear differences between normal and ovarian cancer serum, with GD1(36:1) significantly decreased as previously.

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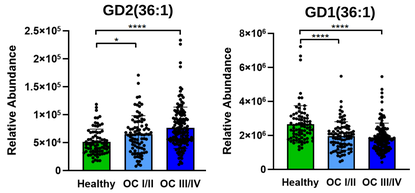
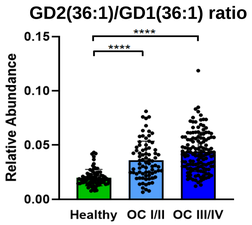
Alterations in GD1 and GD2 point to perturbations in ganglioside metabolism

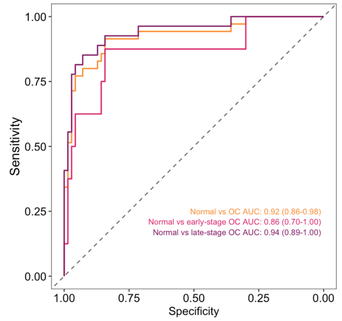


Previously observed alterations in GD2(36:1) and GD1(36:1) were replicated in our second cohort.



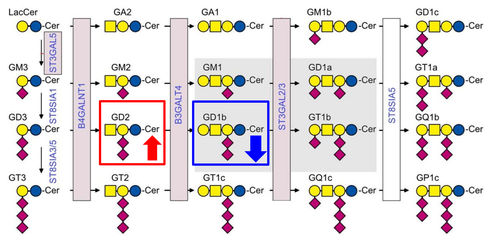
We then interrogated differences in the ratio of GD2(36:1) to GD1(36:1), which amplified fold change differences and significance when comparing normal vs. early- and late-stage OC.

GD2(36:1)/GD1(36:1) distinguishes normal from OC serum



ROC curve analysis from our first cohort shows the ratio of GD2(36:1) to GD1(36:1) distinguishes normal from OC subjects with an AUC of 92% (95CI 86-98%), as well as early- and late-stage OC with AUCs of 86% and 94%, respectively.

Increased GD2/GD1 ratio suggests a reduction in B3GALT4 enzymatic activity



A decrease in GD1 and increase in GD2, resulting in an increased GD2(36:1)/GD1(36:1) ratio, suggests the activity of B3GALT4, the enzyme converting GD2 to GD1, is perturbed.

Figure: Sipione et al. Gangliosides in the Brain: Physiology, Pathophysiology and Therapeutic Applications. Front. Neurosci. 2020

Results

We observed alterations in the ganglioside profile in both early- and late-stage OC serum compared with healthy individuals, most notably a decrease in GD1(36:1) and an increase in GD2(36:1). As GD1 is converted to GD2 by B3GALT4, we explored differences in the ratio of GD2(36:1) to GD1(36:1) and found a significant increase for early- and late-stage OC across both cohorts. Receiver operating characteristic (ROC) curve analysis found the ratio of GD2(36:1) to GD1(36:1) distinguishes normal from OC subjects with an area under curve (AUC) of 92% (95CI 86-98%), and early- and late-stage cancers with AUCs of 86% and 94%, respectively.

Conclusion

We identified an altered ganglioside profile in early- and late-stage OC serum when compared with healthy individuals. Notably, the ratio GD2(36:1)/GD1(36:1) acts as a sensitive and specific signature that can distinguish early- and late-stage OC from healthy patients. Alterations in this ratio suggest perturbation of the enzyme B3GALT4. Future studies will validate and investigate these findings in a population with signs and symptoms of OC. Our goal is to create a robust statistical model that effectively differentiates non-cancer from OC subjects with high sensitivity and specificity.

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