Sex-associated Biomarker Signatures in Malignant Glioma: Insights from a Proteomics Study

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Introduction

- Malignant gliomas, including glioblastomas (GBM), are aggressive primary brain tumors with poor prognosis and limited diagnostic and therapeutic tools.
- Sex is an important factor in glioma biology: **incidence of GBM is lower in females than in males and typically females show better outcomes, survival, and responses to therapy**[1].
- However, the molecular mechanisms underlying these sex-associated differences remain underexplored, limiting the integration of sex as a biological variable in clinical practice.
- Previously, using FYR Bio's proprietary SPARCs technology, we were able to capture extracellular vesicle (EV) subpopulations with unique characteristics allowing us to differentiate malignant brain tumors from benign tumors and healthy samples.
- In this study, we applied the same **SPARC** technology to isolate tumor-derived (using **Tumor SPARCs** panel) and neuro-derived (using **Neuro SPARCs** panel) EV subpopulations from plasma and performed proteomic profiling to uncover sex-associated biomarkers in malignant glioma patients.

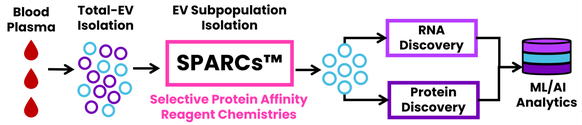

Figure 1. EV- Omics Discovery Platform

Study Design

Table 1. Patients included in the study

	N	Age				Sex		Race		
		<51	51-61	61-70	71-80	Male	Female	White	Asian	Black
Glioma	52	18	14	18	2	23	29	51	1	0
Healthy	38	13	12	11	2	21	17	34	0	4

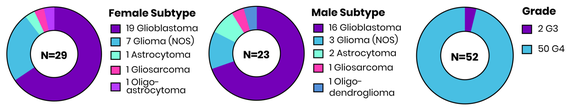

Figure 2. The distribution of glioma subtype by sex

EV Characterization

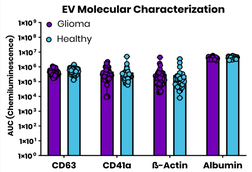

Figure 3: EV molecular characterization across Brain Cancer and Healthy samples

EV marker protein composition was confirmed via Capillary Electrophoresis (CE) Western Blot. EV markers **CD63** (MISEV Cat1a), **ß-Actin** (MISEV Cat2b), **CD41a** (MISEV Cat1b), and **Albumin** (MISEV Cat3a), confirm the presence of EVs as well as the common plasma co-isolate **Albumin**.

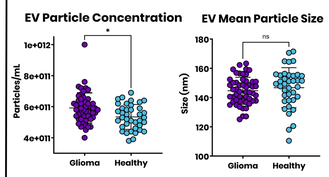

Figure 4: EV particle concentration and mean particle size distribution across groups

Nanoparticle Tracking Analysis (NTA) yielded an average of $5.7x10^{11}$ particles/mL plasma with a mean diameter of 145.7nm. **There was a significant difference in concentration between Brain Cancer and Healthy donors** (p=0.036, Kruskal-Wallis test), consistent with literature citing elevated circulating EV content in Brain Cancer patient plasma.

EV-Proteomics

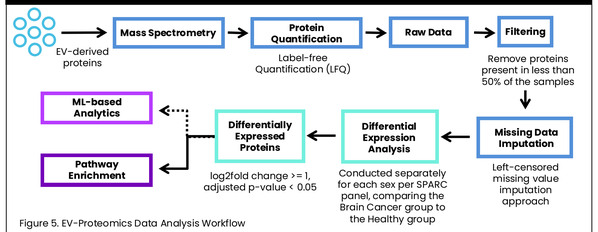

Figure 5. EV-Proteomics Data Analysis Workflow

Differentially Expressed Proteins

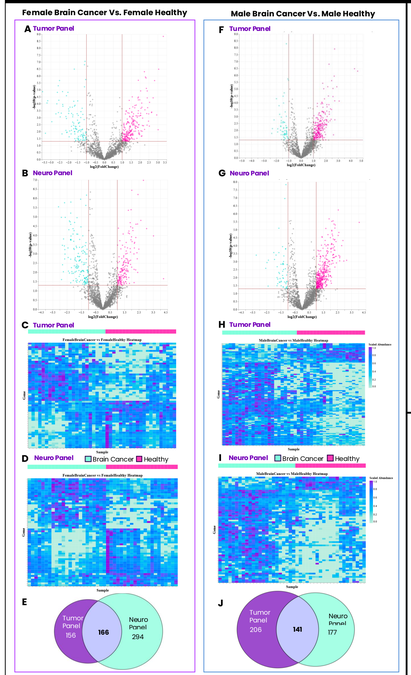

Figure 6. Differentially expressed proteins identified between Brain Cancer and Healthy samples in each sex using Tumor and Neuro SPARC panels. Volcano plots showing significantly up-regulated (pink) and down-regulated(teal) proteins in Brain Cancer samples compared to Healthy samples in each sex: Female (A,B), Male (F,G)(log2fold change >= |1|, p-value < 0.05). Heatmaps of differentially expressed proteins (log2fold change >= |2|, adj. p-value < 0.01) in Female (C,D) and Male (H,I) comparisons (Brain Cancer Vs. Healthy). Venn diagrams showing differentially expressed proteins identified by both panels in each sex: Female (E), Male (J) when comparing Brain Cancer samples to Healthy samples.

Table 2. Differentially expressed proteins (DEPs) identified between Brain Cancer and Healthy groups in each sex using the Tumor (A) and Neuro (B) Panels.

A) Tumor Panel

Comparison	DEPs	Up	Down
Male Brain Cancer Vs. Healthy	100	81	19
Female Brain Cancer Vs. Healthy	155	100	55

B) Neuro Panel

Comparison	DEPs	Up	Down
Male Brain Cancer Vs. Healthy	198	169	29
Female Brain Cancer Vs. Healthy	142	66	76

*Significance criteria: |Log 2-fold change| >= 1 adjusted p-value (Benjamini-Hochberg) < 0.05

Differentially Expressed Proteins (DEPs) Unique to Each Sex

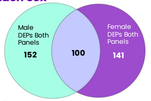

Figure 7. Venn diagram showing common and unique DEPs between Brain Cancer and Healthy groups identified in each sex with both SPARCs panels combined.

- DEPs unique to the **Female** Brain Cancer vs. Healthy comparisons (both SPARCs) were enriched for Gene Ontology (GO) terms associated with **Calcium ion binding** and **proteosome complex**.
- In the DEPs unique to the **Male** comparisons, we observed an enrichment for GO terms associated with **ribosome/translation and amino acid metabolism**.

Pathway Enrichment

Tumor Panel

- In general, the Tumor Panel shows pathways broadly associated with **tumor growth, proteostasis and adaptive and innate immune responses.**
- In the female cohort, we observed **proliferative, proteostatic, and adaptive-immune programs.**
- In the male cohort, we observed **coordinated activation of translation and innate inflammatory programs** indicating a **biosynthetically intense, pro-inflammatory tumor environment.**

Neuro Panel

- In general, the Neuro Panel shows pathways involved in **brain microenvironment and mitochondrial signal.**
- In the female we observed **coordinated suppression of mitochondrial and respiratory pathways**, accompanied by reduced Neutrophil Extracellular Trap (NET) signaling, indicating a **metabolically conserved yet immunologically restrained neural microenvironment**.
- In males, we observed a coordinated activation of **protein synthesis and innate immune pathways**, reflecting a **biosynthetically active and pro-inflammatory neural microenvironment**.

Sex-associated signatures

✓ Female: **Controlled tumor growth under metabolic conservation and adaptive immune programs.**
✓ Male: **Hyperactive, pro-inflammatory, and stress-adaptive programs.**

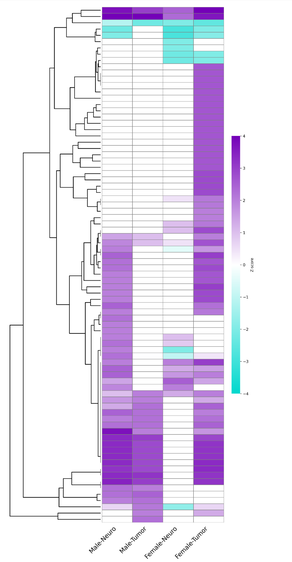

Figure 8. Tile plot shows a comparison of pathway enrichment analysis conducted using Qiagen's Ingenuity Pathway Analysis (IPA) software on each sex specific SPARC panel run. Differentially expressed proteins between Brain Cancer and Heathy groups in each sex obtained from Tumor and Neuro panels were used in this analysis. A total of 82 pathways met the significance criteria (|Z-score| > 2, -log10(adj. p-value) > 1.3)

Candidate Protein Features

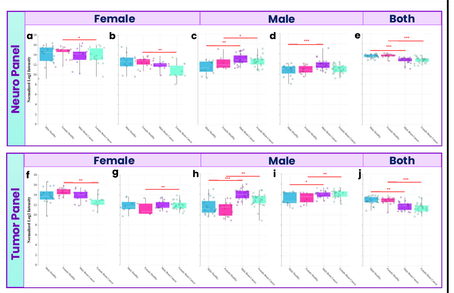

Figure 9. Distribution of protein intensity across candidate protein features identified from DEPs and machine learning using the **Neuro Panel (top)** and the **Tumor Panel (bottom)**. Figures **a, b, f,** and **g** represent features for differentiating **Female Brain Cancer** from Healthy samples. Figures **c, d, h,** and **i** represent candidate features for differentiating **Male Brain Cancer** from Healthy samples. Figures **e** and **j** in each panel represent features for differentiating Brain Cancer from Healthy in general.

- Candidate features were identified based on differential expression, pathway enrichment, and ML models.
- Features for identifying Brain Cancer in the **Females** were associated with **oxidative phosphorylation, proteosome, and adaptive immune response.**
- Features for identifying **Male** Brain Cancer were associated with **protein synthesis and pro-inflammatory responses.**

Machine Learning Analysis

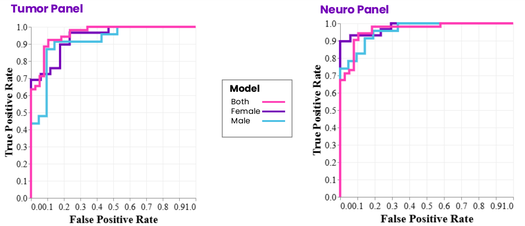

Figure 10. Receiver Operating Characteristic (ROC) curves comparing the performance of multiple classification models. The area under each curve (AUC) indicates overall model discriminative ability to differentiate Brain Cancer from Healthy

Table 3. Performance metrics for leading machine learning models (Brain Cancer Vs. Healthy)

SPARC	Study	AUC	Accuracy (Specificity = 0.90)	Sensitivity (Specificity = 0.90)	Sensitivity (Specificity = 0.99)
Neuro Panel	Female	0.99	0.91	0.91	0.90
Neuro Panel	Both	0.96	0.90	0.90	0.74
Neuro Panel	Male	0.93	0.84	0.80	0.67
Tumor Panel	Both	0.97	0.90	0.88	0.64
Tumor Panel	Female	0.94	0.78	0.74	0.69
Tumor Panel	Male	0.90	0.68	0.52	0.44

A supervised learning algorithm was used to differentiate Brain Cancer samples from Healthy. A 5-fold cross-validation strategy was used for measuring performance. All models show strong discriminative ability, with AUC values ranging from 0.90 to 0.99. Specificity was locked at 0.9 and 0.99 to calculate sensitivity and accuracy. **The Neuro Panel outperforms the Tumor Panel across metrics, especially in the Female cohort**. Female-specific or combined models tend to achieve the highest AUC and accuracy, suggesting potential sex-based biological differences that improve model precision when analyzed separately.

Conclusions & Implications

- The characterization of EV subpopulations in the plasma of Brain Cancer patients provides opportunities for non-invasive disease detection and improved understanding **of sex-associated differences in malignant glioma.**
- Distinct DEPs across both SPARC panels (Figures 6–7) and between male and female comparisons highlight **sex-specific molecular signatures**.
- In **Female glioma**, pathway enrichment (Figure 8) indicates **rapid but controlled tumor growth under metabolic conservation** and **enrichment of adaptive immune responses** (consistent with previous studies[2,3]).
- In **male glioma**, we observe a stronger **activation of pro-inflammatory and innate immune pathways**, aligning with **greater T-cell exhaustion** reported in males.[4]
- Further, ML models trained on female-specific datasets outperform models combining both sexes, which underscores the importance sex-aware approaches in glioma diagnosis.
- Overall, our results from the sex-stratified EV proteomics provide evidence supporting the need for sex-aware experimental design and tailored therapeutic strategies in glioma.

References

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