#125 Ultra High-Plex Spatial Proteogenomic Investigation of Giant Cell Glioblastoma Multiforme Immune Infiltrates Reveals Distinct Protein and RNA Expression Profiles

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Introduction

Spatially resolved, multiplex proteomic and transcriptomic technologies have revolutionized and redefined the approaches to complex biological questions pertaining to tissue heterogeneity, tumor microenvironments, cellular interactions, cellular diversity, and therapeutic response. Most spatial technologies yield single analyte proteomic or transcriptomic datasets from separate formalin-fixed paraffin-embedded (FFPE) tissues sections. Multiple studies have demonstrated poor correlation between RNA expression and protein abundance owing to transcriptional and translational regulation, target turnover, and post-translational protein modifications. Accurately measuring RNA and protein simultaneously within a single tissue section with distinct spatial context is critical to a more complete biological understanding of cellular interactions and activities. Such multimodal omic datasets of protein and DNA or RNA have been termed "spatial proteogenomics".

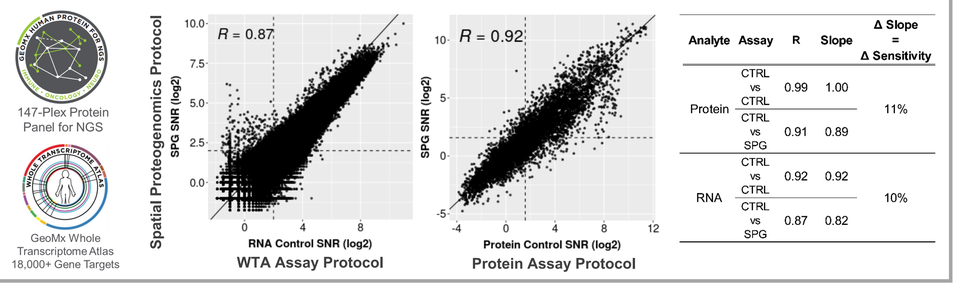
Here we present a novel spatial proteogenomic (SPG) assay on the GeoMx® Digital Spatial Profiler platform with NGS readout that enables ultra high-plex digital quantitation of proteins (147-plex) and RNA (whole transcriptome, > 18,000-plex) from a single FFPE sample. We used the SPG assay to interrogate 23 different glioblastoma multiforme samples across 4 pathologies. We observed clustering of both RNA and protein based on cancer pathology and anatomic location. The in-depth investigation of giant cell glioblastoma multiforme (gcGBM) revealed distinct protein and RNA expression profiles compared to that of glioblastoma multiforme (GBM). Spatial proteogenomics allowed simultaneous interrogation of critical protein post-translational modifications alongside whole transcriptomic profiles within the same distinct cellular neighborhoods.

We observed >2-fold higher protein expression levels of phospho-GSK3β (Ser9) in gcGBM compared to GBM. Inactivation of GSK3β through phosphorylation has been shown to enhance proliferation of GBM cells. We also observed differential protein expression phosphorylated Tau variants. Phospho-Thr231 Tau was >2-fold higher in GBM compared to gcGBM. Associated with neurodegenerative Alzheimer's disease, changes in Tau expression and phosphorylation have also been observed in glioblastoma. Our study exemplifies the utility of the SPG assay in expanding our understanding of glioblastoma multiforme molecular pathology.

GeoMx Spatial Proteogenomics Assay (SPG) - High-plex Protein and RNA Detection On A Single Slide

A

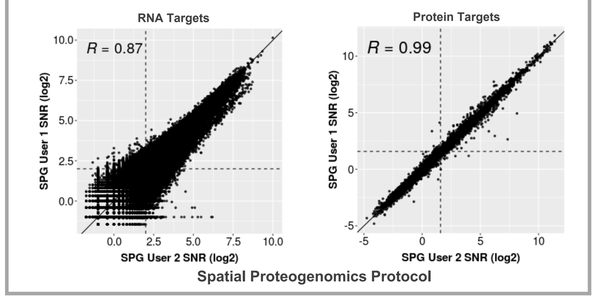
With highly concordant performance to the single analyte GeoMx WTA or NGS Protein Assays, GeoMx Spatial Proteogenomic Assay eliminates variation from differing cell populations across serial slides.



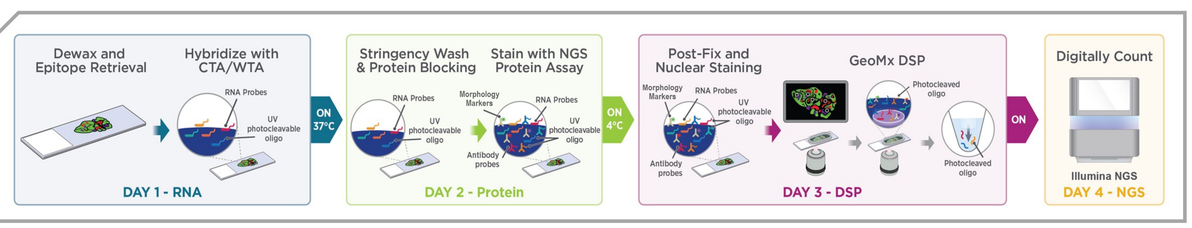
Analyte	Assay	R	Slope	Δ Slope = Δ Sensitivity
Protein	CTRL vs CTRL	0.99	1.00	11%
	CTRL vs SPG	0.91	0.89	
RNA	CTRL vs CTRL	0.92	0.92	10%
	CTRL vs SPG	0.87	0.82	

B

User friendly GeoMx Spatial Proteogenomics Protocol results in highly concordant results from slide to slide & user to user.



C

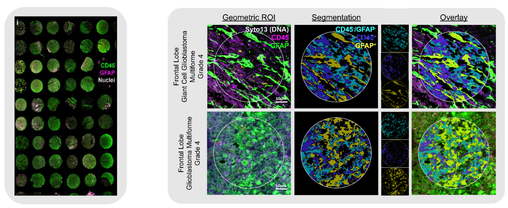


GeoMx Spatial Proteogenomic Workflow. The assay takes 4 days from slide prep to data analysis and is only compatible with NGS readout. Prior to staining, samples are subjected to a two-step epitope retrieval process involving HIER under basic conditions followed by proteolytic digestion with 0.1 µg/mL ProK.

GeoMx Spatial Proteogenomic Evaluation of Grade 4 Glioblastoma

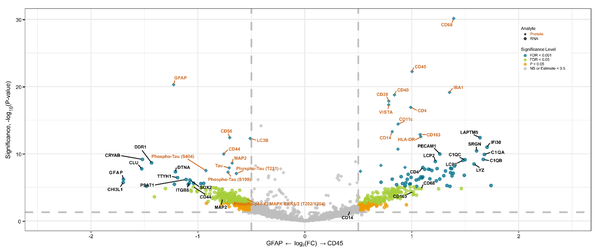
A

Able to optically dissect immune and astrocytic/tumor cells in various GBM subtypes.



Glioblastoma Grade 4	
Sample Type	FFPE Array
Sample cores per slide	42
ROI/AOI	76/224
RNA Data Points	4,214,560
Protein Data points	32,928
Total Data Points	4.25 million

B



Observed differential expression of key immune or tumor associated proteins and RNA.

C

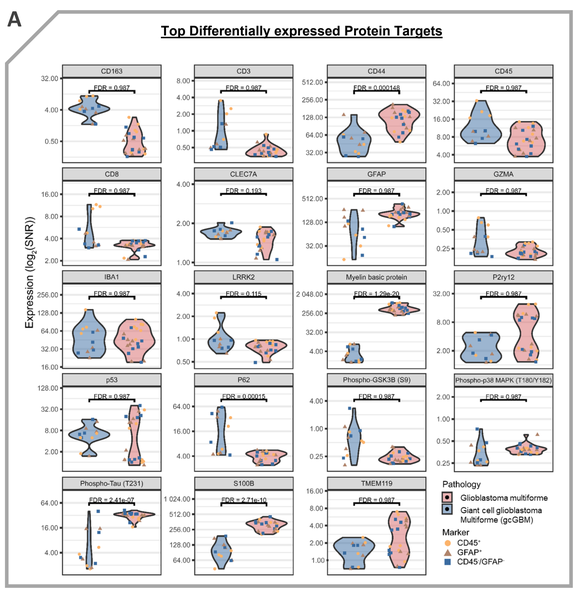
Clustering based on pathology and tumor anatomic location

Spatial Proteogenomic exploration of Glioblastoma multiforme Grade 4. **(A)** 42 cores across 23 distinct sample sources and 4 different (grade 4) GBM sub-types. ROI (300 µm) were segmented into CD45$^+$ (Immune), GFAP$^+$ (Astrocyte/Tumor), or CD45$^-$/GFAP$^-$. Statistics from a single slide and single GeoMx Spatial Proteogenomic run. (**B**) Combined volcano plot resulting from the differential expression analysis between GFAP and CD45 enriched segments for RNA (●) and protein (♦) analytes. Targets with significantly differential expression are highlighted either orange (P-value < 0.05), green (FDR < 0.05) or blue (FDR < 0.01); whereas targets in grey show no significant difference in expression. (**C**) Unsupervised hierarchical clustering analysis of detected RNA and protein.

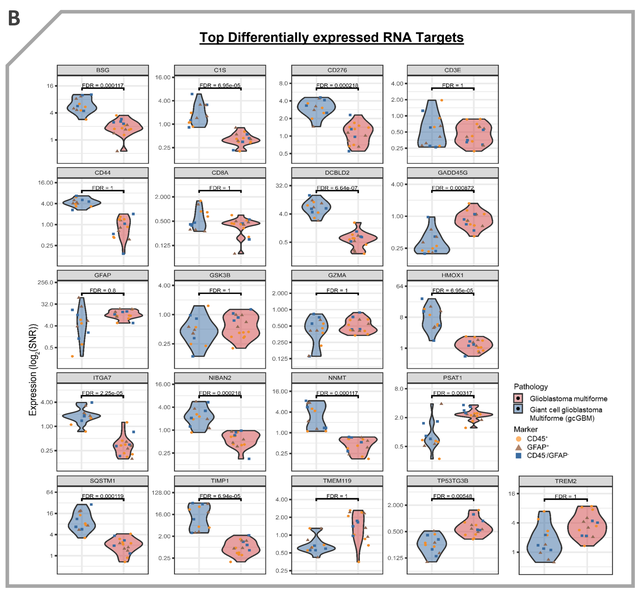
Distinct Protein and RNA Expression Profiles Between Left Frontal Lobe gcGBM and GBM

Able to identify differentially expressed RNA and protein (including phosphorylated) targets between subtypes in both immune and astrocytic/tumor cells.

A



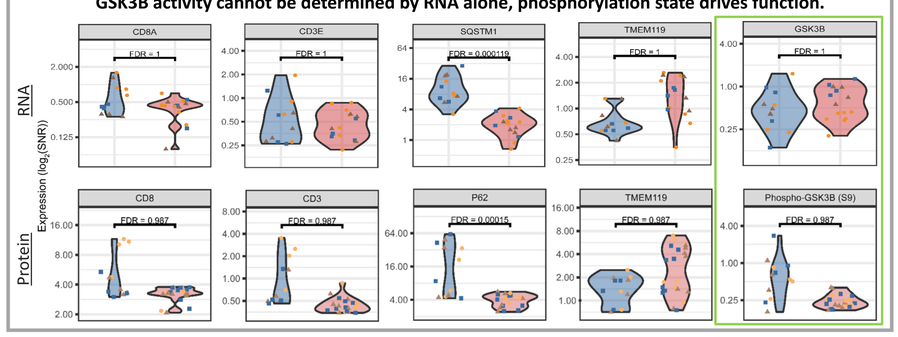
B



Spatial Proteogenomic exploration of Frontal Lobe gcGBM and GBM; all Grade 4. Analysis was restricted to one gcGBM and two GBM patient cases. Differentially expressed (**A**) Protein and (**B**) RNA Targets. **(C)** Key target RNA and Protein expression profiles in gcGBM compared to GBM (**D**) Combined volcano plot resulting from the differential expression analysis between GBM and gcGBM for all AOIs for RNA (●) and protein (♦) analytes.

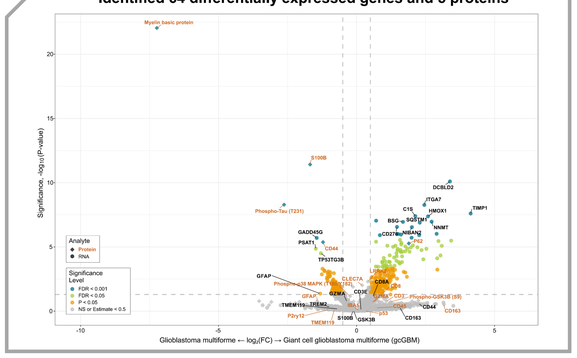
C

Protein expression patterns indicate increased immune infiltration and activity in gcGBM. GSK3B activity cannot be determined by RNA alone, phosphorylation state drives function.



D

Identified 64 differentially expressed genes and 5 proteins



Conclusion

The GeoMx Spatial Proteogenomic Assay allows for the > 18,000-plex RNA and 147-plex protein co-detection of proteins and RNA from a single FFPE slide.

- > 18,000-plex RNA and 147-plex protein characterization of limited and precious biological samples
- Multi-omic (RNA + Protein) profiling from identical spatially resolved cell populations
- Eliminates technical variability between –omic datasets

Spatial proteogenomic investigation of immune/tumor microenvironments of multiple GBM subtypes offers a rich discovery space.

- Optically dissect distinct astrocytic/tumor and immune cells in various GBM subtypes
- Distinct clustering of both RNA and protein observed, based on pathology and anatomic location
- Identify differentially expressed protein and RNA targets between GBM and gcGBM
- Identify critical post translational modifications (PTMs) that drive function, not able to be determined by RNA alone

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