

#0141 Ultra High-Plex Spatial Proteogenomic Investigation of Giant Cell Glioblastoma Multiforme Immune Infiltrates Reveals Distinct Protein and RNA Expression Profiles

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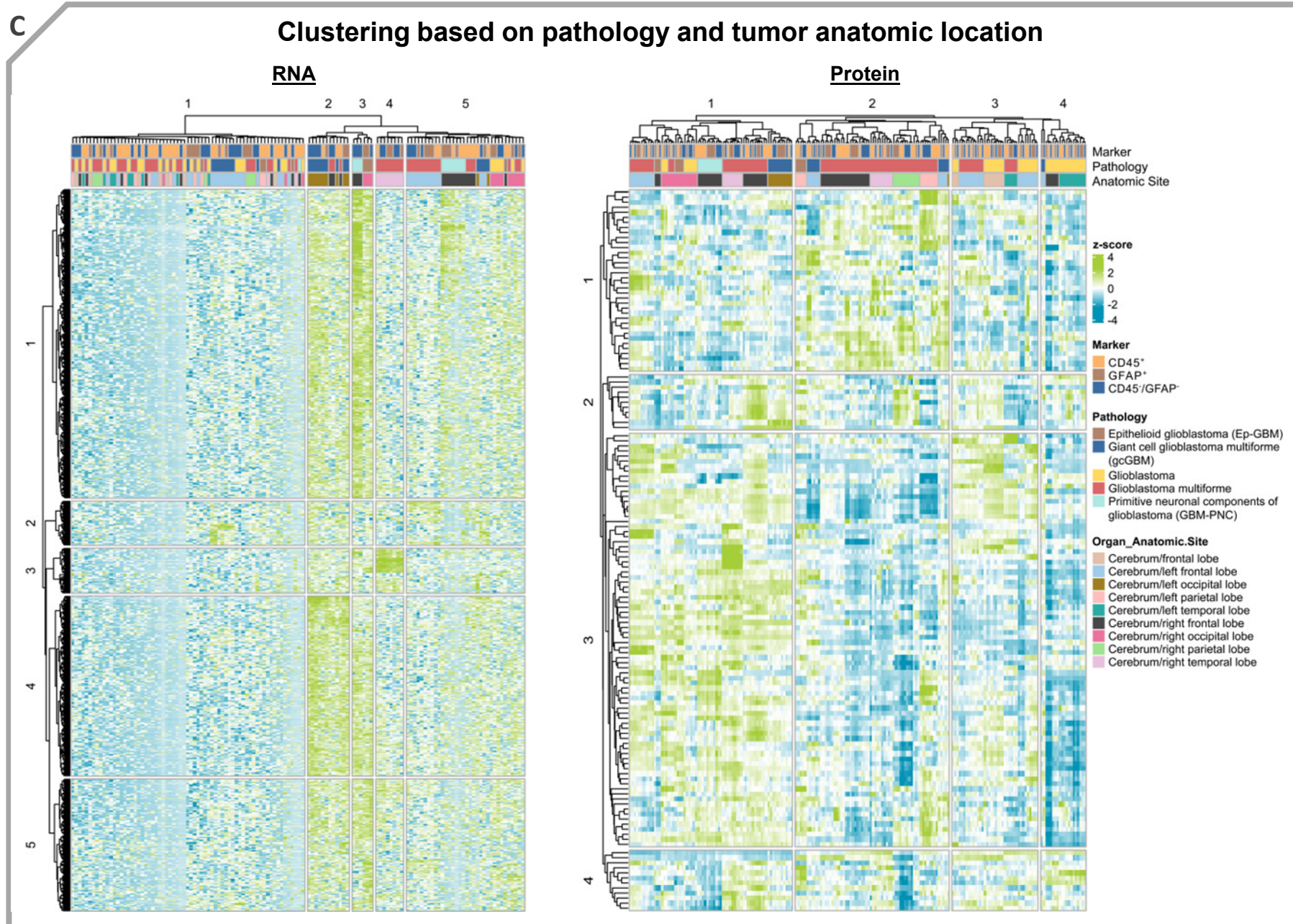
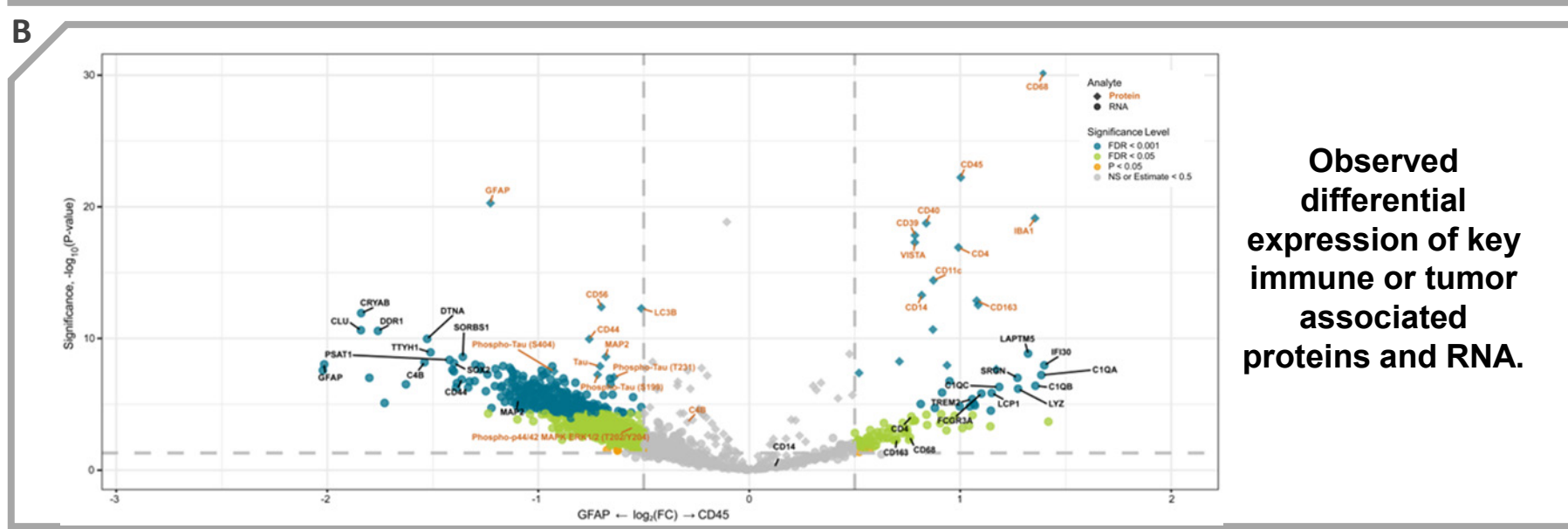
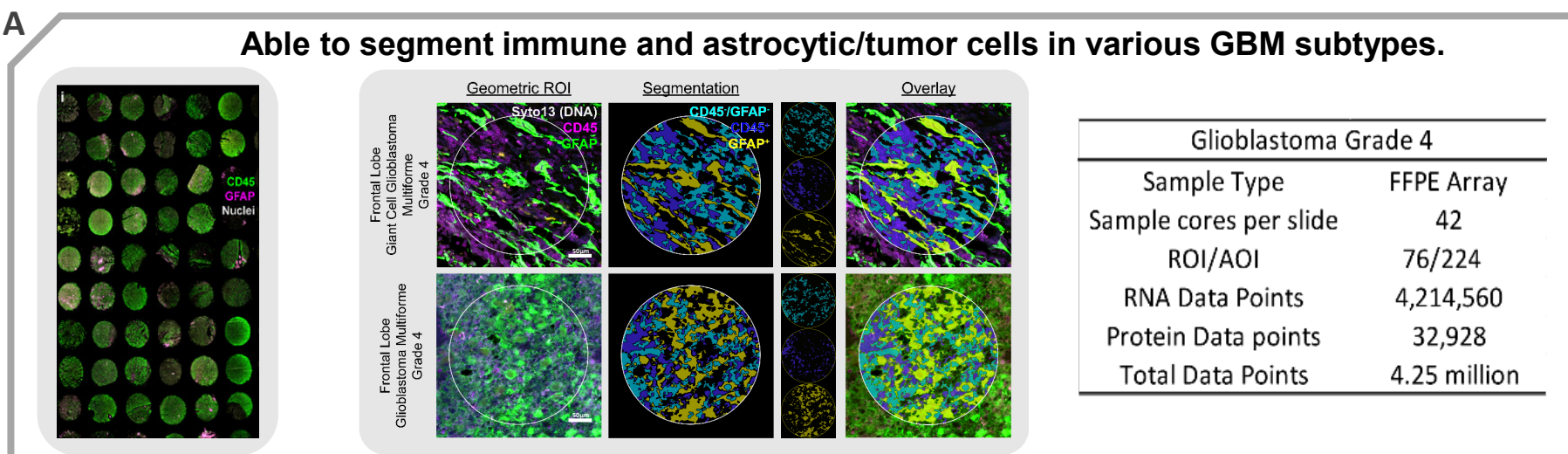
Introduction

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 quantification 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.

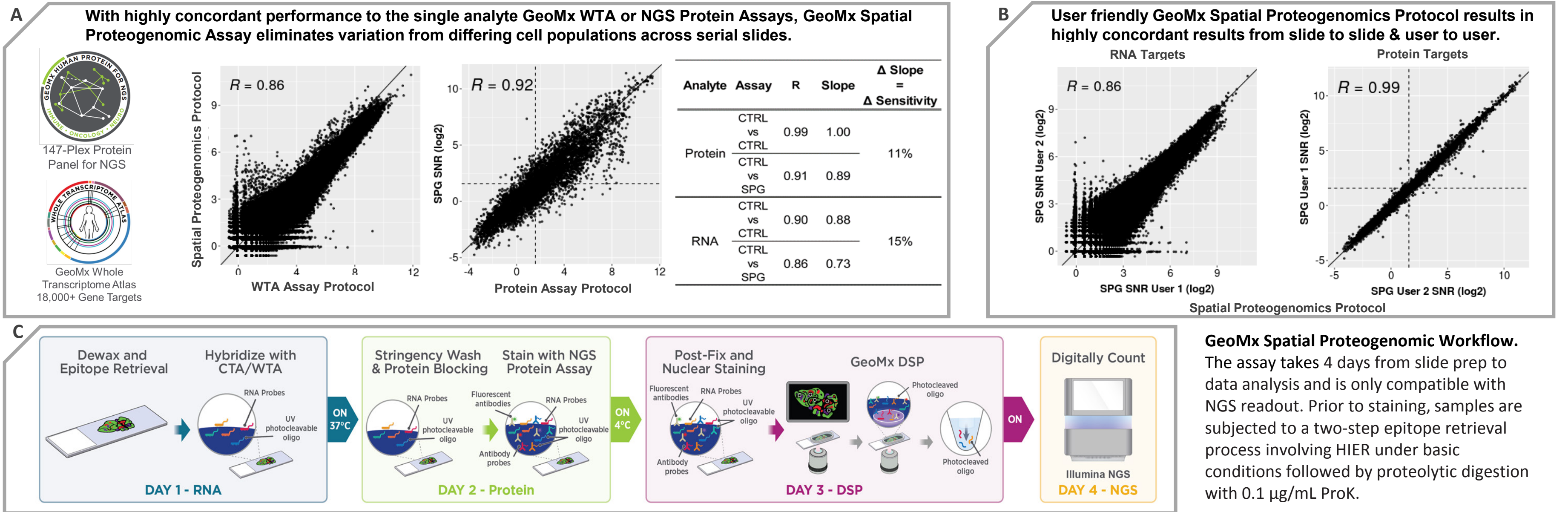
We observed >2-fold higher protein expression levels of phospho-GSK3β (Ser9) in gcGBM compared to GBM. Inactivation of GSK3β through phosphorylation has been shown to enhance proliferation of GBM cells. We also observed differential protein expression phosphorylated Tau variants. Phospho-Thr231 Tau was >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 Proteogenomic Evaluation of Grade 4 Glioblastoma



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.

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



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.

