Advancing Glycomics in High Grade Gliomas: A MIBI-TOF and MALDI Imaging Integrated Approach

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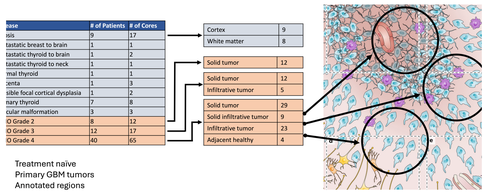
INTRODUCTION

High grade gliomas (HGG) are the most common and lethal primary tumor of the brain. Even with an aggressive standard of care regimen of surgery, radiation, and chemotherapy, the rate of 5-year survival is just 4%. While the advent of immunotherapy has improved prognosis in a variety of malignancies, its efficacy in HGG has been disappointing. HGG lesions can consist of tumor-dense regions with pseudopalisading necrotic foci, immune-dense regions enriched for MDSCs, and marginal regions where in many cases no clear boundary between healthy and malignant glial cells can be delineated. Furthermore, post-translational regulation through glycosylation provides another layer of complexity that had not been appreciated until recently. The work proposed here will provide a deeper understanding of the cellular states and transitions of the HGG TME that promote disease progression.

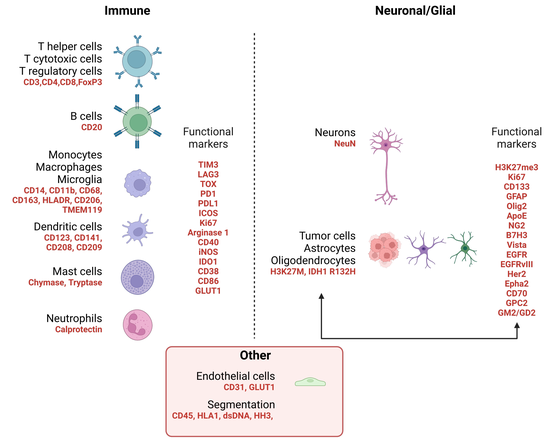


ase	# of Patients	# of Cores
ma	9	17
tastatic breast to brain	1	1
tastatic thyroid to brain	1	2
tastatic thyroid to neck	1	1
mal thyroid	1	1
centa	1	3
sible focal cortical dysplasia	1	2
nary thyroid	7	8
cular malformation	3	3
O Grade 2	8	12
O Grade 3	12	17
O Grade 4	40	65

Cortex	9
White matter	8
Solid tumor	12
Solid tumor	12
Infiltrative tumor	5
Solid tumor	29
Solid infiltrative tumor	9
Infiltrative tumor	23
Adjacent healthy	4

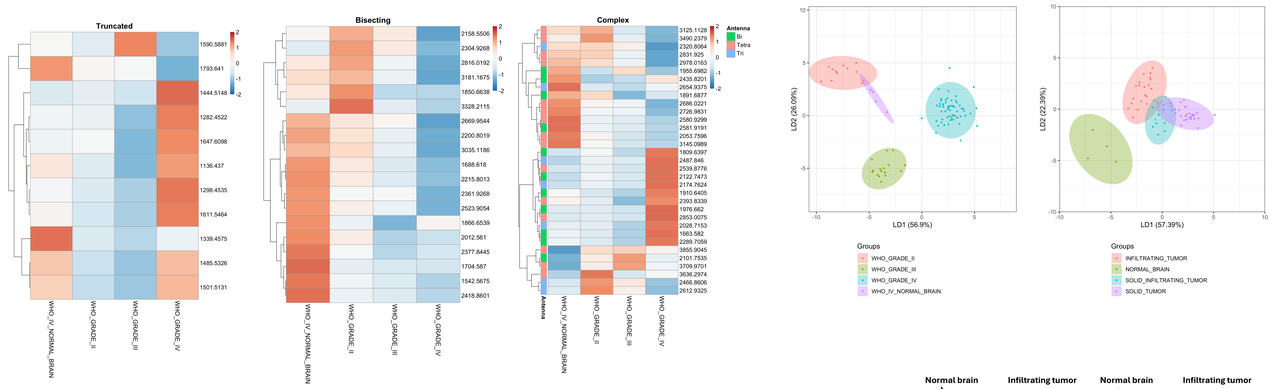
Summary of Stanford glioma cohort samples with grade and anatomical location

RESULTS



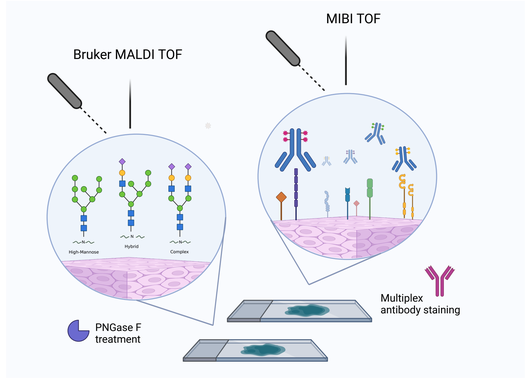
Top Multiplexed antibody panel for Glioma analysis using MIBI-TOF. ***Bottom*** HGG (glioblastoma multiforme, GBM) sample stained with above panel, acquired using MIBI-TOF

Our work is focused on high-dimensional, high-resolution imaging and molecular analysis to understand how the glioma TME changes with transition from low to high-grade lesions. We analyze archival FFPE tissue using two orthogonal mass spectrometry imaging modalities—highly multiplexed immunohistochemical imaging by MIBI-TOF and de novo spatial N-glycomics by MALDI. These complementary analyses will each reveal unique aspects of state transitions occurring in the glioma microenvironment upon disease severity and recurrence. MIBI-TOF subcellular imaging is used to capture the composition, functional state, and spatial organization from a cell-centric perspective. However, highly multiplexed imaging assays like MIBI that rely on antibodies for protein detection are poorly suited for capturing the biochemical diversity of glycans. Thus, N-glycomics with MALDI imaging is used to map the spatial distribution of N-glycans in the glioma TME without the use of targeted probes.



Left, top Z score heat map of truncated, bisecting, and complex N-glycans with tumor (grade II, III, IV) and adjacent non tumor regions ***Right, top*** LDA analysis showing complete separation of tumor grades and gradual separation of tumor regions. ***Right, bottom*** Glioblastoma infiltrating tumor (cerebral cortex, right parietal) N-glycan H4N5 expression, mass 1704.6 m/z, at 10 µm resolution in adjacent non tumor region along with serial section hematoxylin and eosin stain.

METHODS



Multiplexed Ion Beam Imaging by Time of Flight (MIBI-TOF) uses mass spectrometry and metal-labeled antibodies to visualize up to 50 proteins at once at subcellular resolution to comprehensively identify the lineage, function, and spatial location of every cell in a single intact tumor tissue section.

De novo glycomics using MALDI mass spectrometry is used to map the spatial distribution of glycans in serial sections adjacent to those used for MIBI-TOF. In this approach, each tissue is sprayed with a thin layer of recombinant peptide N-glycosidase F enzyme (PNGaseF) that cleaves protein-bound *N*-glycans. Because the tissue is not immersed in a buffer, these glycans do not diffuse away from their point of origin and remain spatially fixed. Subsequent imaging mass spectrometry by Bruker MALDI-TOF MS permits detection and spatial registration of these species with 10 µm resolution. This approach is completely compatible with FFPE tissues and does not require any purification or enrichment steps prior to analysis. Annotated peak libraries are used to identify TME glycans. Each peak definition provides details on glycan composition.

CONCLUSION

These complementary analyses each reveal unique aspects of state transitions occurring in the glioma microenvironment. Preliminary data illustrates how integration of MALDI N-glycomics imaging, and spatial cell phenotypic data from the same samples can be used to reveal previously unknown spatial features. Here, we show examples of N-glycan features that drive separation between tumor grade and anatomical regions, offering insights into the biology of glioma progression. Furthermore, this integrated approach allows for a deeper understanding of the cellular interactions and molecular mechanisms that define these transitions.

The authors declare the following competing financial interest(s): M.A. and S.C.B. are inventors of patent US20150287578A1. M.A. and S.C.B. are board members and shareholders in IonPath Inc.