Utilizing Mass Spectrometry Imaging to Identify Potential N-Glycan Prognostic Biomarkers for Temozolomide Resistance in Glioblastoma Multiforme Tissues

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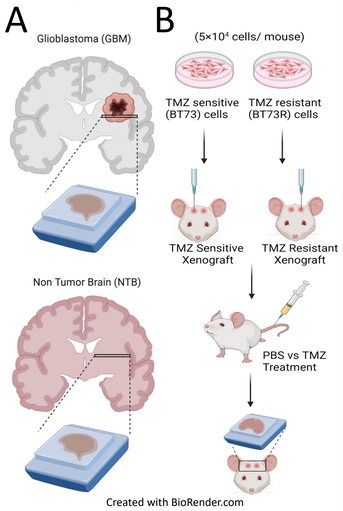
OVERVIEW

Glioblastoma (GBM) is the most prevalent malignant brain tumor among adults with a grim 5-year survival rate of 8-15 months. Despite Temozolomide (TMZ) being the primary treatment, more than half of patients do not respond, emphasizing the urgent need for new insights.

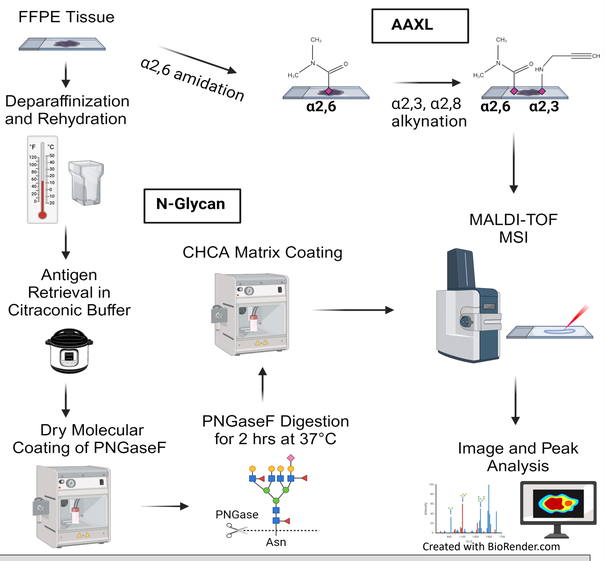
Using Matrix Assisted Laser Desorption/Ionization mass spectrometry imaging (MALDI MSI), we identify distinct N-glycosylation changes in GBM and N-glycans associated with TMZ resistance.

Our investigation includes matched primary and recurrent GBM tissues (n=4), alongside non-tumor brain (NTB) samples from epileptic patients as controls (n=2) (A).

Additionally, a SCID mouse model was used to elucidate the effects of TMZ on brain tumor initiating cells (BTICs), implanting patient derived BT73 and BT73R cells into mouse brains and administering TMZ treatment according to established protocols (n=3) (B).



METHOD



ACKNOWLEDGMENTS

This research was funded by NSERC. There are no COI.

MALDI images were created using SCILS software.

RESULTS

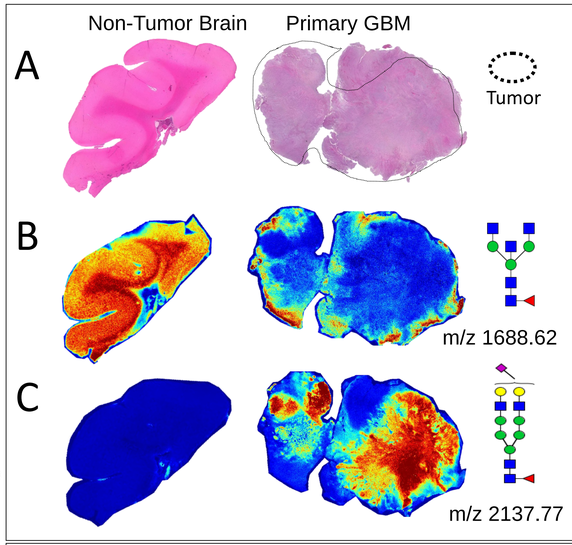


Figure 1. Unique N-glycan pattern in GBM compared to Non-tumor Brain.

Using pathology annotated Hematoxylin and Eosin (H&E) adjacent tissues sections to guide our analysis (Figure 1A), we identified 64 unique N-glycans in primary GBM and NTB tissues. Notably, NTB samples exhibited higher levels of bisecting structures as represented by m/z 1688 (Hex3dHex1HexNAc5m/z) compared to GBM (Figure 1B). To investigate linkage-specific changes in sialylated N-glycans, we performed an amidation-amidation reactions using AAXL bioorthogonal click chemistry[1] to differentiate α2,6 from α2,3-linked N-linked sialic acids. The α2,3 sialylated biantennary N-lycan at 2137 m/z (Hex5HexNAc4NeuAc1 + 2Na) exhibited significantly higher intensity in the GBM sample compared to NTB samples (Figure 1C).

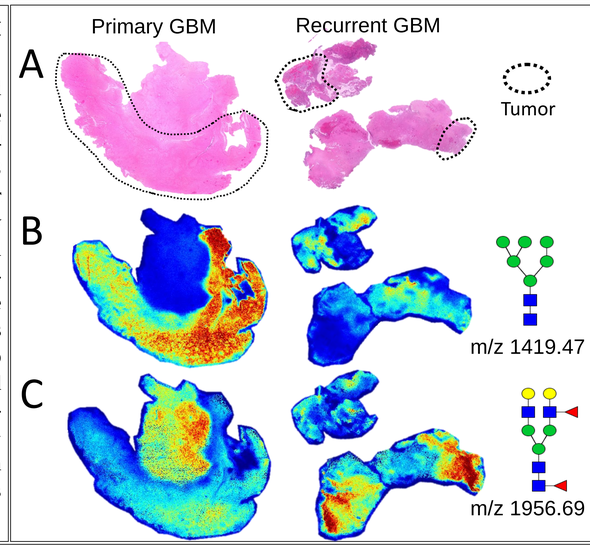


Figure 2. Unique N-glycan pattern in primary and recurrent GBM.

Using pathology-annotated H&E tissue sections as guides (Figure 2A), we compared the relative intensity of the 64 identified unique N-glycans from Figure 1 in primary and recurrent matched GBM tissues. High mannose m/z 1419 (Hex6HexNAc2) exhibited higher intensity in primary GBM compared to recurrent GBM, while fucosylated m/z 1956 (Hex5dHex2HexNAc4) (Figure 2B) showed elevated intensity in recurrent GBM samples (Figure 2C). However, analysis of the 64 unique N-glycans across primary and recurrent GBM tissues did not reveal consistent patterns within either group, underscoring the heterogeneous nature previously documented in GBM[2].

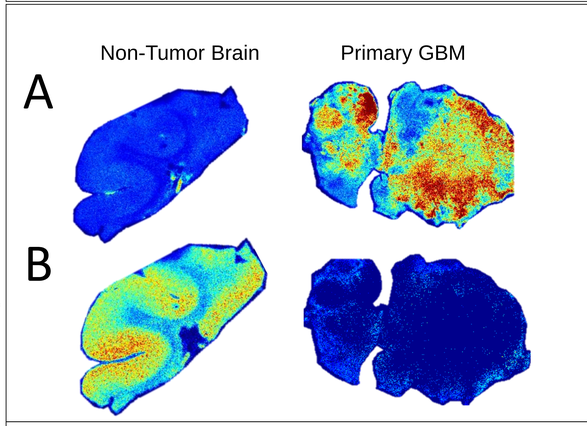


Figure 3. Unique N-glycan pattern in primary GBM also seen in BTIC xenograft tissues.

To investigate glycosylation alterations in GBM and the impact of TMZ treatment, we compared de-identified GBM samples with mouse xenograft GBM tissues to ensure consistency in observed trends. Core fucosylated N-glycans (such as m/z 2174 Hex6dHex1HexNAc5 + 1Na) were found at higher intensities in GBM tissue and present in both TMZ-sensitive and TMZ-resistant xenograft GBM tissues (Figure 3A). Additionally, we identified specific fucosylated N-glycans exclusively present in NTB samples (2508 Hex5dHex3HexNAc6) (Figure 3B). These results highlight the distinctive N-glycan profiles distinguishing controls from GBM samples, while also emphasizing the similarity of fucose signatures between primary and xenografted GBM tumors.

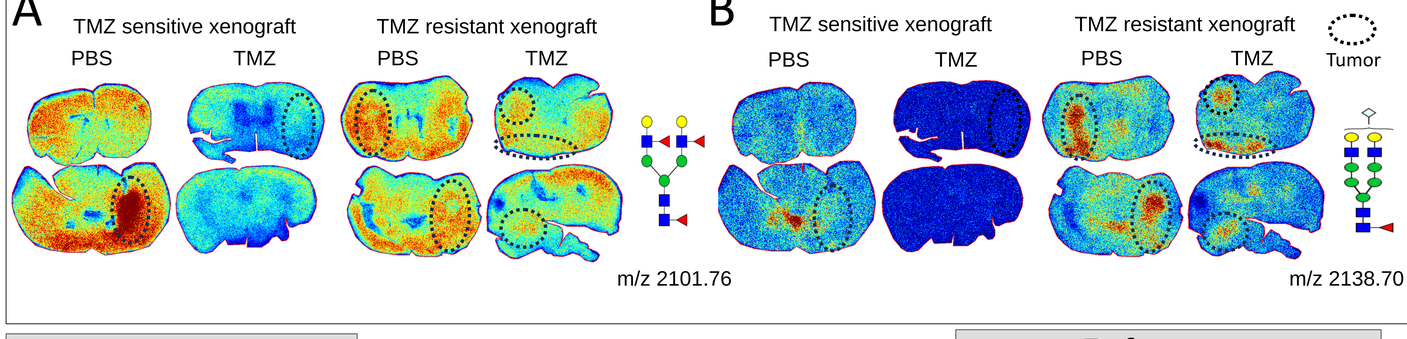


Figure 4. Unique N-glycan pattern TMZ sensitive vs TMZ resistant xenograft

We identified 82 unique N-glycans within both TMZ-sensitive and TMZ-resistant xenograft models. The N-glycan m/z 2101 (Hex5dHex3HexNAc4) is predominantly observed in PBS-treated TMZ-sensitive tissue and showed decreased intensity post-TMZ treatment in TMZ-sensitive tissue and in TMZ-resistant xenografts (Figure 4A). Conversely, the sialylated N-glycan with m/z 2138 (Hex5HexNAc4NeuAc1 + 2Na) was absent in TMZ-sensitive xenografts but exhibited high intensity in TMZ-resistant xenografts, remaining unaffected by TMZ treatment, indicating its potential as a biomarker for TMZ resistance (Figure 4B).

CONCLUSION & FUTURE DIRECTIONS

This study unveils the complex interplay of glycosylation patterns, tumor recurrence, and TMZ resistance in GBM. These findings not only offer potential prognostic insights but also pave the way for personalized diagnostics and targeted therapies in GBM.

References

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