Metabolomic differentiation of tumor core versus edge in glioma

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OBJECTIVE Gliomas exhibit high intratumor and interpatient heterogeneity. Recently, it has been shown that the microenvironment and phenotype differ significantly between the glioma core (inner) and edge (infiltrating) regions. This proof-of-concept study differentiates metabolic signatures associated with these regions, with the potential for prognosis and targeted therapy that could improve surgical outcomes.

METHODS Paired glioma core and infiltrating edge samples were obtained from 27 patients after craniotomy. Liquid-liquid metabolite extraction was performed on the samples and metabolomic data were obtained via 2D liquid chromatography–mass spectrometry/mass spectrometry. To gauge the potential of metabolomics to identify clinically relevant predictors of survival from tumor core versus edge tissues, a boosted generalized linear machine learning model was used to predict metabolomic profiles associated with O6-methylguanine DNA methyltransferase (MGMT) promoter methylation.

RESULTS A panel of 66 (of 168) metabolites was found to significantly differ between glioma core and edge regions (p ≤ 0.05). Top metabolites with significantly different relative abundances included DL-alanine, creatine, cystathionine, nicotinamide, and D-pantothenic acid. Significant metabolic pathways identified by quantitative enrichment analysis included glycerophospholipid metabolism; butanoate metabolism; cysteine and methionine metabolism; glycine, serine, alanine, and threonine metabolism; purine metabolism; nicotinate and nicotinamide metabolism; and pantothenate and coenzyme A biosynthesis. The machine learning model using 4 key metabolites each within core and edge tissue specimens predicted MGMT promoter methylation status, with AUROCEdge = 0.960 and AUROCCore = 0.941. Top metabolites associated with MGMT status in the core samples included hydroxyhexanoylcarnitine, spermine, succinic anhydride, and pantothenic acid, and in the edge samples metabolites included 5-cytidine monophosphate, pantothenic acid, itaconic acid, and uridine.

CONCLUSIONS Key metabolic differences are identified between core and edge tissue in glioma and, furthermore, demonstrate the potential for machine learning to provide insight into potential prognostic and therapeutic targets.

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KEYWORDS glioma; glioblastoma; metabolomics; MGMT promoter methylation; tumor core; tumor edge; machine learning

Tumor heterogeneity in glioblastoma (GBM) can be described by two major regions, the core (inner) and edge (infiltrating), which harbor unique environments that underlie the progression of GBM and are associated with promalignant processes including phenotypic transitions and heightened invasiveness, respectively. Recent work has also suggested that metabolic changes manifest spatially across the tumor and are essential mediators of tumor adaptation and therapy evasion. The edge demonstrates an increased expression of genes associated with fatty acid metabolism as well as abundance of acylcarnitines, but also has high glucose metabolism, whereas the core tends to exhibit more metabolic plasticity and reliance on amino acid metabolism. Although the contributions of metabolic changes to GBM progression are clear, the specific metabolites and metabolic pathways responsible for these alterations are poorly understood.

Metabolomics provides a potential advantage in its...
ability to describe functional changes to the cells that complement the transcriptional and proteomic profiles of the tumor. Early reports in metabolomics studies have shown that unique metabolic signatures can be identified between low-grade and high-grade gliomas via tissue and serum from patients. One study that performed gas chromatography/mass spectrometry with CSF found citric and isocitric acid to have higher levels in GBM than in grades 1–3 gliomas. Metabolomics studies have traditionally used human body fluids such as urine, blood, CSF, and other sera to characterize cancer. However, these approaches cannot resolve differences within the tumor. Therefore, studying the cancer tissue directly, rather than the biofluids, may provide more accurate insight into intratumoral metabolic differences, and yield detailed information about tumor microenvironment heterogeneity and potential cell-scale mechanisms. Very recently, key metabolic differences between adult glioma subtypes were identified from resected tissue.

A variety of techniques have been applied for analysis of metabolomic data, including network-oriented techniques such as principal network analysis, and data-driven methods such as statistical modeling and machine learning, as recently reviewed. In parallel, substantial work has focused on the application of machine learning—including radiomics and radiogenomics—to GBM imaging data, for distinguishing true progression from pseudoprogression, to evaluate intratumoral heterogeneity, and to analyze imaging with deep learning. In this study, core and edge GBM tumor tissue pairs obtained from craniotomy and resection underwent liquid–liquid metabolite extraction, and the metabolomic profiles of the core and edge regions were contrasted. Differences in these profiles are important because the core is typically removed in surgery whereas the edge is left behind. Furthermore, machine learning was applied to evaluate the potential of metabolomics to identify clinically relevant predictors of survival from tumor core versus edge tissues. Here, O’-methylguanine DNA methyltransferase (MGMT) promoter methylation status was chosen as an example for evaluation, with the goal being to demonstrate the capability to provide insight into potential prognostic and therapeutic targets. MGMT status has been associated with the prognosis of patients with GBM, in whom methylation typically improves overall survival. MGMT is a DNA repair protein and is associated with alkylating chemotherapeutic agents such as temozolomide (TMZ). It is currently unknown how MGMT status manifests in the metabolome of GBM tissue. Applying machine learning to deepen the understanding of GBM intratissue metabolomic heterogeneity and identifying key differences would be relevant for future customization of treatment.

Methods

Patient Sample Tissue and Gene Status Collection

Informed consent was obtained to participate in this study. All specimens were collected following approved internal review board protocols at University of Louisville Hospital from patients with known or suspected brain tumors. Given that brain cancer affects both women and men, samples from both were collected. Samples were collected by the clinical team, who were blinded to the research analysis. Patient information was deidentified by the clinical team before evaluation by the research team. IDH-R132H mutation was detected by immunohistochemistry and MGMT promoter methylation by next-generation sequencing (Neogenomics Inc.).

Patient Sample Tissue Processing for Metabolomics

Samples of 27 GBM tumor core and edge pairs obtained at separate times from patients immediately following craniotomy, for a total of 54 samples, were received from the University of Louisville Brown Cancer Center Biorepository. Core samples were collected from the contrast-enhancing portion of the tumor (Fig. 1). Infiltrating edge samples were collected from T2/FLAIR hyperintense surrounding tissue (Fig. 2). For each core–edge pair following craniotomy, the two sections were immediately placed into separate 15-mL centrifuge tubes containing 5 mL of nonsupplemented RPMI medium and then transferred to the laboratory for processing. Tissue sections were placed in separate sterile 1.5-mL microcentrifuge tubes with tweezers. All tissue handling was performed under a biosafety cabinet in a sterile environment. Phosphate-buffered saline (1 mL) was added to each tube, and tubes were centrifuged at 250g for 5 minutes. After supernatant was aspirated, this washing step was repeated for both samples. Cold acetonitrile (500 μL) was added to each sample after the second supernatant was aspirated; samples were then homogenized with a handheld pellet mixer (VWR 47747–370) by grinding the sample against the microcentrifuge tube wall. Next, 375 μL of nuclease-free water and 250 μL of chloroform were added to each tube, followed by vortexing for 10–15 seconds. A final 20-minute centrifugation at 664g was completed to separate polar, protein, and lipid layers. For each sample, polar and protein layers were pipetted into 1.5-mL microcentrifuge tubes, whereas lipid layers were pipetted into 2.0-mL glass screw top vials, and polar layers were lyophilized.

2D Liquid Chromatography–Mass Spectrometry/Mass Spectrometry Sample Processing

Postlyophilization, 2D liquid chromatography–mass spectrometry/mass spectrometry (2DLC-MS/MS) was performed by the Center for Regulatory and Environmental Analytical Metabolomics (CREAM) at the University of Louisville. Each dried sample was dissolved in 200 μL of 50% acetonitrile and vigorously vortexed for 3 minutes. After centrifugation at 14,000 rpm and 4°C for 20 minutes, 100 μL was collected for further analysis. All samples were analyzed on a Thermo Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer coupled with a Thermo DIONEX UltiMate 3000 HPLC system (Thermo Fisher Scientific). The LC system is equipped with a reversed phase column (RPC; Waters Acquity UPLC HSS T3 column, 2.1 × 150 mm, 1.8 μm) and a hydrophilic interaction liquid chromatography column (HILIC; Millipore SeQuant ZIC-cHILIC column, 2.1 × 150 mm, 3 μm). The two chromatographic columns were configured to form a parallel 2DLC-MS system. Mobile phase A was water.
**FIG. 1.** Intraoperative 3D navigation view obtained using MRI of a T1 postcontrast 4-panel view. Images demonstrate a representative core biopsy location within the contrast-enhancing portion of the tumor.

**FIG. 2.** Intraoperative 3D navigation view obtained using MRI of a T1 postcontrast 4-panel view. Images demonstrate representative edge biopsy location outside the contrast-enhancing portion of the tumor within the tumor (infiltrating) edge.
with 0.1% formic acid for RPC, and 35% acetonitrile with 10 mM ammonium acetate (pH adjusted to 3.25 with acetic acid) for HILIC. Mobile phase B was acetonitrile with 0.1% formic acid for both RPC and HILIC. The RPC gradient was 0% B, hold for 6.0 minutes; 6.1 to 14 minutes, 0% B to 28% B; 14.1 to 16 minutes, 28% B to 50% B; 16.1 to 20.0 minutes, 50% B to 100% B, hold for 1.0 minute; 21.0 to 21.1 minutes, 100% B to 0% B, hold for 11.9 minutes. The HILIC gradient was 0% B, 95% B; 1.4 to 8.3 minutes, 95% B to 0% B, hold for 2.7 minutes; 11.0 to 11.5 minutes, 0% B to 95% B, hold for 11.5 minutes. The flow rate was 0.35 mL/min for RPC or 0.3 mL/min for HILIC. Column temperature was 40°C for both RPC and HILIC. Samples were first analyzed in a random order in positive (+) and negative (−) modes to obtain full MS data for metabolite quantification. For metabolite identification, a pooled sample of each group was analyzed by 2DLC-MS/MS in positive and negative modes at three collision energies (20, 40, and 60 eV).

2DLC-MS/MS Data Analysis

A web-based platform (XCMS, RRID:SCR_015538; xcmsonline.scripps.edu) was used for spectrum deconvolution, and MetSign software was used for metabolite identification, cross-sample peak list alignment, normalization, and statistical analysis. To identify metabolites, 2DLC-MS/MS data were first matched to an in-house database that contains parent ion m/z, MS/MS spectra, and retention time of authentic standards. The threshold for spectral similarity was set at ≥ 0.4, whereas thresholds of retention time difference and m/z variation window were set at ≤ 0.15 minutes and ≤ 5 ppm, respectively. 2DLC-MS/MS data without a match with the metabolites in the in-house database were further analyzed using Compound Discoverer software version 2.0 (Thermo Fisher Scientific), where the MS/MS spectra similarity score threshold was set at ≥ 40 with a maximum score of 100. This analysis yielded the intensity peaks for individual metabolites identified in each sample.

Data Preprocessing

Data preprocessing was performed using R programming language version 4.1.0. The metabolite intensity peaks from 2DLC-MS/MS were imported from an Excel (Microsoft Corp.) file. Metabolites absent in 30% or more of the samples were excluded, resulting in 168 unique metabolomic signatures detected across all analytical batches (Supplementary Fig. 1). Data were preprocessed by log transformation and MS total useful signal normalization—both commonly used in metabolomics—and centering. The log transform was applied to minimize heteroscedasticity. MS total useful signal normalization, especially used for liquid chromatography, sums ion MS signals that are common among all samples to develop a normalization factor. Data were centered around the mean to focus on the differences between the data. Given that data appeared to be missing at random (Supplementary Fig. 1), imputation was performed to handle missing values by using Bayesian principal component analysis. Data from all 27 patients were obtained across two separate analytical batches. Batch correction was performed by the Center statistical model on positive and negative ion mode data sets separately (Supplementary Fig. 2).

Statistical Analyses

Paired and unpaired statistical analyses were performed in R version 4.1.0. The Shapiro test was used to determine normality; the t-test or Wilcoxon rank-sum test (Mann-Whitney U-test) was used to determine significant differences between relative abundance of metabolites. A partial least squares discriminant analysis (PLS-DA) was performed with function plsda frommdatools library. A heat map of preprocessed data was generated with the heatmap.2 function from gplots library.

Classification Model

A machine learning model (glmboost) was trained to discriminate between MGMT promoter methylation—positive and negative samples by using tumor core and edge metabolomic data independently. A rigorous combination of feature selection and cross-validation was performed to prevent overfitting. Test set validation was achieved with fivefold cross-validation, performed with 20 iterations of random subsampling. Results were obtained as the average across all folds and iterations. Classification performance metrics (area under the receiver operating characteristic curve [AUROC]; F1) were calculated as the averages across all folds and resampling iterations. Forward feature selection was performed after ranking features by variable importance and training/validating models on feature subsets 2–16. Across all feature subsets, a single optimal trained model was identified for the tumor core and edge. Variable importance was calculated as the absolute value of the coefficients of the tuned model, using function varImp from package caret. Model hyperparameters mstop (number of boosting iterations) and prune (Akaike information criterion [AIC] prune Boolean) were tuned with R package caret (version 6.0–93), where prune = “no” and mstop = 450 and 150 for models trained with core and edge data, respectively.

Metabolic Network Visualization and Quantitative Enrichment Analysis

MetaboAnalyst 5.0 (RRID:SCR_015539; www.metaboanalyst.ca/) was used to obtain chemical structure classes and quantitative enrichment analysis (QEA). The Kyoto Encyclopedia of Genes and Genomes (KEGG) (RRID:SCR_012773; www.kegg.jp/) database was accessed (March 2023). Metabolic networks were visualized with Cytoscape 3.8.2 (RRID:SCR_003032; cytoscape.org/) and MetScape 3.1.3 (RRID:SCR_014687; metscape.ncbi.org/).

Results

Patient Characteristics

Of 27 patients (Table 1), 23 were White and 4 were of Black or African American ethnicity. The average age at tissue collection was 52 years (SD ± 17.7 years). Twenty-two patients had WHO grade 4 GBM; the remainder had...
Table 1. Characteristics in 27 patients with gliomas

<table>
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<tr>
<th>Case No.</th>
<th>Ethnicity</th>
<th>Age (yrs) at Tissue Collection</th>
<th>WHO Grade</th>
<th>Histology</th>
<th>IDH1 Mutation</th>
<th>MGMT Promoter Methylation</th>
<th>Disease Type</th>
<th>Prior Therapies</th>
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AA = anaplastic astrocytoma; NA = not available; OA = oligoastrocytoma; OD = oligodendroglioma.

Metabolic Differences in Tumor Core and Edge Tissue

PLS-DA separated tumor core and edge samples significantly ($P_{overlap} = 5.37e-11$) (Fig. 3). A volcano plot of upregulated and downregulated metabolites between tumor core and edge is also shown. Metabolites with significant differences in relative abundance between paired tumor core and edge samples are shown in Supplementary Table 1, with the top 20 (by lowest $p$ value) compared between core and edge in Fig. 4. Top metabolites upregulated in edge samples were DL-alanine ($p = 6.41e-07$), creatine ($p = 7.85e-07$), cystathionine ($p = 1.02e-06$), nicotinamide ($p = 1.31e-06$), D-pantothenic acid ($p = 1.47e-06$), and 3-hydroxy-3-[(3-methylbutanoyl)oxy]-4-(trimethylammonio)butanoate ($p = 3.08e-06$). Top metabolites upregulated in core samples were 2-oxobutyric acid, uric acid, threonine, and N1,N12-diacetyspermine. Comparison of remaining significant metabolites between core and edge samples are shown in Supplementary Figs. 3–5. Furthermore, 5 metabolites were identified with significantly different relative abundance between patients with MGMT promoter methylation–positive or –negative status, including higher hydroxyhexanoylcarnitine and lower spermine in core, and both higher itaconic acid and pantothenic acid in edge samples (Supplementary Fig. 6, Supplementary Table 2).

Classifying Samples by MGMT Promoter Methylation Status

PLS-DA score plots reveal significant separation between MGMT promoter methylation–negative and –positive samples using both tumor core ($P_{overlap} = 3.18e-05$) and edge ($P_{overlap} = 3.11e-04$) metabolomic data (Fig. 5). This indicates that classification via machine learning could provide insight. A classification model was trained and validated as described in Fig. 6. Excellent classification performance (Fig. 5C) was achieved using the top 4 metabolomic features as ranked by variable importance (Supplementary Table 3), with AUROC$_{Core} = 0.941$ (95% CI 0.905–0.967) and AUROC$_{Edge} = 0.960$ (95% CI 0.937–0.982) for core and edge samples, respectively. Model
performance across all feature subsets (2–10 or 2–11 for core and edge, respectively) is summarized in Supplementary Fig. 7. For both the tumor core and edge, AUROC increases initially and begins to decline after 4 features, and F1 also shows a decline after 4 features. Overall, these results indicate that the features ranked by glmboost variable importance were effective at filtering noise out of the data sets.

Quantitative Enrichment Analysis

The relative proportion of chemical structure main classes within tumor core and edge samples across both analytical batches is presented in Supplementary Fig. 8. The largest classes included amino acids and peptides, fatty acids and conjugates, fatty esters, purines, and tricarboxylic acids (TCAs). The most significantly enriched metabolic pathways between core and edge (Supplementary Fig. 9), where the enrichment ratio is the number of observed hits divided by the number of expected hits, were glycine, serine, and threonine metabolism; nicotinate and nicotinamide metabolism; pantothenate and coenzyme A (CoA) metabolism; glutathione metabolism; and cysteine and methionine metabolism. Pathway maps of significant metabolic pathways identified by QEA for tumor core versus edge are shown in Fig. 7 and Supplementary Fig. 10.

Discussion

Little is known about differences in metabolic landscapes in spatially distinct core and edge (infiltrating) regions of gliomas. This proof-of-concept study identified 66 unique metabolomic signatures that differed significantly between paired core and edge human glioma specimens (Fig. 3). A total of 5 metabolites were found to be significantly different in terms of relative abundance between MGMT promoter methylation–positive and –negative patients for core as well as for edge (Supplementary Fig. 6). Furthermore, a machine learning model (glmboost) was able to accurately classify MGMT status by using metabolomic data from tumor core and edge samples (Fig. 5C).

The TCA cycle occurs within mitochondria in order to produce adenosine triphosphate. Glutamic acid, a significantly differentiated metabolite found between core and edge regions (Supplementary Fig. 3), is referred to synonymously as glutamate (Glu), the form of glutamic acid found within the body. Glu is highly involved in energy metabolism. It is a key factor in glutaminolysis, a process that prepares substrates for the TCA cycle and serves an important role in cancer metabolism. An increased level of Glu in the edge relative to the core, therefore, reflects the actively invasive nature of the edge. Astrocytes have been shown to uptake extra Glu produced by glioma cells, however, if the astrocyte/glioma cell ratio is too small, as in higher-grade glioma, when the ratio approximates 0.5:1, then cell death may ensue. Glutamine, a driver of invasion in GBM, is abundant in the brain and is a direct precursor to Glu. Gliomas uptake glutamine through the upregulation of transporters of glutamine and Glu, and its deprivation is known to slow GBM tumor growth. The results suggest that increased Glu detected in the infiltrating tumor edge may be due to increased glutamine uptake and conversion. Furthermore, as a TCA cycle intermediate, pyruvate (Supplementary Table 3) is associated with increased levels of Glu, citrate, malate, and aspartate. Creatine...
4), associated with amino acid metabolism, has been noted as an energy metabolism marker.\textsuperscript{27} It is therefore unsurprising that creatine and Glu were found to be upregulated in the edge (infiltrating) tumor regions. Pantothenic acid, also known as vitamin B5 and pantothenate, is an essential cofactor involved in many metabolic reactions and is the precursor of CoA.\textsuperscript{28,29} CoA is an essential component of acetyl-CoA, which serves many roles and participates in the TCA cycle and B-oxidation (lipid catabolism).\textsuperscript{30} D-pantothenic acid, detected as a positive ion, was upregulated in tumor edge samples (Fig. 4) and pantothenic acid, detected as a negative ion, was also included in the top 10 metabolites as ranked by the classification model in which either tumor core or edge data were used (Supplementary Table 3). Succinic anhydride, the acid anhydride of succinic acid, was also upregulated in tumor edge samples (Supplementary Fig. 4), consistent with the finding that succinate accumulation enables tumor cells to be more aggressive.\textsuperscript{31} Uric acid (i.e., urate) was among the top 20 most significant metabolites in terms of relative abundance between

![FIG. 4. Relative abundance box plots of the top 20 metabolites differentiating between tumor core and tumor edge samples, ranked by p value. Paired t-tests or Wilcoxon rank-sum tests were performed, depending on the normality of the data. POS and NEG prefixes on metabolite names indicate whether the ion was detected in positive or negative ion mode. Values on the y-axis represent signal intensity postprocessing.](image-url)
core and edge tissue (Fig. 4), and was also within the top 10 most important metabolites for predicting MGMT promoter methylation status by using core tissue data (Supplementary Table 3). Uric acid levels may correlate with cancer progression. Deng et al. found that patients with type 2 diabetes who had higher levels of serum uric acid (≥ 5.0 mg/dL) experienced significantly higher rates of cancer than those with lower levels (< 3.0 mg/dL). It remains unclear why uric acid levels would be increased in the core of gliomas. Nicotinamide, a form of vitamin B3, was the fourth most significantly upregulated metabolite in the infiltrating tumor edge (Fig. 4), and nicotinate and nicotinamide metabolism was found to be the second most significantly enriched metabolic pathway (Supplementary Fig. 9). Nicotinamide has been associated with neuronal development, survival, and CNS function, and has implications in neurodegenerative conditions such as Alzheimer’s disease. Nicotinamide N-methyltransferase is known to be overexpressed in GBM and is preferentially expressed by GBM stem cells. Nicotinamide is an important intermediate in the production of nicotinamide adenine dinucleotide (NAD+) and is involved with the main-
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Tenance of NAD and NAD phosphate. Nicotinamide is connected with NAD+ directly via reactions R00110 and R00555 (KEGG) (Supplementary Fig. 10). NAD+ regulates functions for many metabolic pathways, including glycolysis and the TCA cycle. Intracellular NAD(H) levels have been found to control motility and invasion in glioma cells, for which downregulation of nicotinamide phosphoribosyltransferase resulted in limited cell migration compared to controls. Oral supplementation with nicotinamide has been shown to increase blood levels of NAD, suggesting that high levels of nicotinamide may increase the biosynthesis of NAD. It can therefore be hypothesized that increased levels of nicotinamide found in the infiltrating tumor edge may also result in increased NAD levels, suggesting that NAD biosynthesis may reflect intratumoral heterogeneity. Higher NAD in the tumor edge would further support the evidence that NAD levels have an impact on cell motility in glioma.

Purine metabolism (Supplementary Fig. 10), a pathway found to be significantly enriched between core and edge tissue, has recently garnered interest in the study of glioma metabolism. It has been established that brain tumor initiating cells (BTICs) are dependent on the downstream components of de novo purine synthesis. It is known that the GBM tumor edge has a preferential expression of glioma stemlike cell signatures, where CD133 and CD109 have different expression profiles in tumor cells localized at the core and edge regions. Hypoxanthine, which was increased in the tumor edge (Supplementary Fig. 3), has also been found to be elevated in BTICs. Guanosine monophosphate (GMP), which is linked to hypoxanthine through inosine monophosphate and reactions R01134 and R01132 (KEGG) (Supplementary Fig. 10), was also elevated in the tumor edge (Supplementary Fig. 4). GMP has been found to be essential to the maintenance of BTIC proliferation, self-renewal, and tumorigenicity. Taken together, these differences may indicate an increased abundance of this cell phenotype in the tumor edge. GMP was also found to be dramatically decreased in GBM tissue treated with TMZ. Interestingly, uric acid (i.e., urate) was increased in the tumor core (Fig. 4), which may indicate a decreased rate of production of uric acid in the tumor edge from hypoxanthine or xanthine through reactions R01768 and R02107 (KEGG) (Supplementary Fig. 10). The path-

FIG. 7. Pathway maps of significant metabolic pathways identified by QEA for tumor core versus edge, including glycerophospholipid metabolism; butanoate metabolism; cysteine and methionine metabolism; and glycine, serine, alanine, and threonine metabolism. Red source nodes are shown connected to compounds (pink hexagons), enzymes (green squares), genes (purple circles), and reactions (gray diamonds). Upward orange arrows or downward blue arrows underneath each metabolite (red) node indicate whether the metabolite was significantly upregulated or downregulated with respect to the tumor core (e.g., creatine was downregulated in tumor core/upregulated in tumor edge; L-serine was upregulated in tumor core/downregulated in tumor edge).
way analysis further highlights ethanolamine phosphate downregulated in core, indicating reduced lipid metabolism; serine upregulated in core, promoting cell adaptation to hypoxic conditions; decreased cystathionine in core, linked to GBM progression; and methionine higher in core, known to be prevalent in glioma.

Due to the limited sample size in this proof-of-concept study, potential confounding variables consisting of age, histology, IDH1 mutation, MGMT promoter methylation, and primary versus recurrent disease could not be adjusted for (Table 1). Accounting for these variables is planned in the future with a larger sample set. GBM is heterogeneous and consists of multiple cell types, including tumorigenic stem cells, stromal cells, and differentiated bulk tumor cells. The bulk tissue specimen analysis used in this study is useful for determining global characteristics of the broadly defined tissue regions, but more nuanced specimen analyses could be performed—e.g., via single-cell matrix-assisted laser desorption/ionization MS (MALDI-MS). Moreover, platforms such as MetaboAnalyst are limited because not all metabolites have been documented in reference databases; thus, several compounds detected here could not be characterized or classified.

Conclusions

We have presented a proof-of-concept study evaluating metabolic differences in paired tumor core and infiltrating edge glioma samples, and we have shown that unique metabolomic signatures distinguish these two tumor regions. Furthermore, the potential of metabolomics to identify clinically relevant predictors of survival from these regions was evaluated by accurately classifying MGMT promoter methylation status. In the longer term, metabolomics integrated with other analyses such as tumor tissue biophysical characterization might provide a comprehensive view of glioma heterogeneity. Investigation of mechanisms driving tumor aggression and invasion separately within core and edge regions could potentially identify therapeutic targets to minimize recurrence and benefit surgical outcomes.

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References


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**Author Contributions**

Conception and design: Frieboes, Chen, Williams. Acquisition of data: Williams. Analysis and interpretation of data: Frieboes, Baxter, Miller. Drafting the article: Baxter, Miller. Reviewing the article: all authors. Approved the final version of the manuscript: Frieboes. Miller. Approved the final version of the manuscript on behalf of all authors: Frieboes. Statistical analysis: Baxter, Miller. Administrative/technical/material support: Frieboes, Williams. Study supervision: Frieboes, Williams.

**Supplemental Information**

Online-Only Content


**Data Availability**

Data sets have been deposited in Metabolomics Workbench (MWB, RRID:SCR_013794; www.metabolomicsworkbench.org).