Review Article: Metabolomics in High Grade Gliomas

Abstract:
Gliomas are central nervous system (CNS) cancers that are challenging to treat due to their high proliferation and mutation rates. High grade gliomas include grade 3 and grade 4 tumors, which characteristically have a poor prognosis despite advancements in diagnostic methods and therapeutic options. Advances in metabolomics are resulting in more insight as to how cancer modifies the metabolism of the cell and surrounding tissue. Hence, this avenue of research may also emerge as a way to precisely target metabolites unique to gliomas. These biomarkers may provide opportunities for glioma diagnosis, prognosis and future therapeutic intervention.

In this review, we harvest the literature that highlights notable biomolecules in high grade gliomas and promising therapeutic targets and interventions.

Key words: metabolomics, glioma, tumor, brain, cancer, CNS

Introduction
High-grade gliomas (HGGs) include the World Health Organization (WHO) grade 3 and 4 tumors and consist of astrocytoma (IDH-mutant), oligodendroglioma (IDH-mutant and 1p/19q-codeleted), glioblastoma (IDH-wildtype), diffuse hemispheric glioma (H3 G34-mutant and H3 K27-mutant), among other gliomas (Louis et al., 2021). The typical treatment plan includes surgical resection and a combination of chemotherapy and radiotherapy. Despite that, these tumors tend to recur with poor prognosis and limited treatment options.

Metabolomics is the study of endogenous metabolites and low molecular weight molecules (LMWM) and their interactions within a biological sample (Nicholson and Lindon, 2008; Patti et al., 2012). Metabolomics is a discipline integral to advancing scientific understanding and discovery of new, effective clinical diagnoses and interventions. The significance of this discipline has appeared in how we frame and diagnose cancers. Historically, the WHO has classified and diagnosed cancers histogenetically. More recently, in the fifth edition of the WHO classification of tumors of the Central Nervous System (WHO CNS5), cancer taxonomy and diagnosis are layered and involves an integration of molecular features and histology (Louis et al., 2021).
This review describes recent metabolomic studies that pertain to high grade gliomas, spanning metabolic pathways, metabolites, therapeutic interventions, and testing methods.

**Critical metabolic pathways**

In normal mammalian cells, metabolic pathways maintain homeostasis and sustain life. However, cancer cells disrupt and exploit metabolism to promote abnormal growth, survival, and proliferation. The Warburg Effect is a well-documented phenomenon that illustrates metabolic dysregulation (Warburg, 1925; Warburg, 1956). Under typical conditions, glucose is oxidized to pyruvate through glycolysis and mitochondrial oxidation of pyruvate to acetyl-CoA continues to support energy production via the tricarboxylic acid cycle (TCA) and oxidative phosphorylation. In the 1920s, Otto Warburg observed that cancer cells have increased glucose uptake and produce lactate from glucose despite the presence of oxygen and completely from glucose (Warburg, 1925; Warburg, 1956). Under typical conditions, glucose is a phenomenon that illustrates metabolic dysregulation (Warburg, 1925; Warburg, 1956). Increased lactate generation noted in the Warburg effect can be explained, in part, by the activation of the PI3K/AKT/mTOR pathway by mutation in the tumor suppressor, PTEN, and/or functioning mitochondria (Warburg, 1925; Warburg, 1956).

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Glucose

Glucose is the most common energy source for mammalian cells; it can be converted to pyruvate through glycolysis and produced via gluconeogenesis. Glucose uptake and usage is markedly increased in cancer cells (Bao et al., 2019; Ishikawa et al., 1993; Warburg, 1925; Warburg, 1956). Several studies indicate that elevated glucose promotes GBM cell growth, and hyperglycemia is a condition associated with worse outcomes (Bao et al., 2019; Derr et al., 2009; Ishikawa et al., 1993; Tieu et al., 2015).

Lactate

Lactate (2-hydroxypropanoic acid) is produced by lactate dehydrogenase-A (LDH-A) in hypoxic conditions. Despite ample oxygen levels, cancer cells preferentially produce lactate and rely on substrate level phosphorylation for their energy (Seyfried and Shelton, 2010). Studies have shown that lactate has multifactorial roles in tumorigenesis, functioning as a signaling molecule for tumor angiogenesis and contributing to tumor inflammation and growth by attracting macrophages that then secrete cytokines such as IL-23/IL-17 and growth factors (Shime et al., 2008; Sonveaux et al., 2012; Végran et al., 2011). Amongst patients with high grade gliomas undergoing tumor resection, pretreatment serum lactate was found to be a strong prognostic biomarker (Mariappan et al., 2015; Shih et al., 2017). Overexpression of LDH-A has also been reported in GBM cell lines and is associated with increased glycolysis, growth, immune evasion, and decreased cell death (Kahlon et al., 2016; Li et al., 2016).

Isocitrate Dehydrogenase, D-2-hydroxylglutarate

IDH catalyzes the oxidative decarboxylation of isocitrate to α-KG with the reduction of NADP+ to NADPH. IDH1 and 2 are homologous enzymes that differ in that IDH1 is localized to the cytosol while IDH2 resides in the mitochondrial matrix. IDH1 mutations at R132 have been associated with astrocytomas, GBM, and oligodendrogliomas, and IDH2 mutations at R172 have been linked with gliomas (Dang et al., 2010; Yan et al., 2009). IDH1/2 have been linked to enhanced reductive carboxylation in hypoxia for de novo lipogenesis (Metallo et al., 2011). In a study of IDH1-mutant (IDH1-mt) gliomas, one study found decreased triglycerides and sphingolipids and elevated pyruvate entering the TCA cycle in IDH1-mt gliomas compared to IDH-wt (Zhou et al., 2019). As a prognostic marker, several studies have found that IDH1/2 mutations are linked to prolonged patient survival and therapeutic response amongst patients with (Jin and Zhou, 2019; Patra and Hay, 2014). Fatty acid oxidation occurs in multiple organelle types including the mitochondria, peroxisome, and endoplasmic reticulum (Talley and Mohiuddin, 2022). This process generates energy under stress and is linked to cellular respiration and proliferation in malignant gliomas (Lin et al., 2017). Evidence of cancer’s unique energy metabolism and the role of the mitochondria have led some in the scientific community to consider cancer a metabolic disease (Erb et al., 2008; Khan et al., 2021; Kwon et al., 2015; Seyfried and Shelton, 2010; Spratlin et al., 2009).
gliomas and GBM (Cairncross et al., 2014; Chen et al., 2016; Parsons et al., 2008; Yan et al., 2009).

While it is well established that many patients with glioma experience at least one seizure in the course of their illness (Moots et al., 1995; van Breemen et al., 2007), epileptogenicity remains poorly understood. Recent studies have pointed to IDH1/2-mt cancers as vulnerable to higher seizure incidence relative to IDH-wt (Chen et al., 2017; Zhong et al., 2015). This may be due to the production of D-2-hydroxyglutarate (D2HG) via NADPH-dependent reduction of α-KG in IDH1/2-mt cancers (Dang et al., 2010; Lee et al., 2019). α-KG is believed to have anti-epileptic properties (Yamamoto and Mohanan, 2003) and D2HG competitively inhibits α-KG dependent enzymes (Xu et al., 2011) which may ultimately contribute to carcinogenesis and seizures. Additionally, D2HG structurally resembles the excitatory neurotransmitter, glutamate, and D2HG in IDH1-mt gliomas have been associated with seizure incidence due to their agonistic actionon the NMDA receptor (Chen et al., 2017).

Glutamate

Glutamate is a CNS excitatory neurotransmitter. Studies indicate that gliomas release glutamate and this has been implicated in tumor expansion (Takano et al., 2001), cellular edema (Savaskan et al., 2008), and tumor associated excitotoxicity and epilepsy (Buckingham and Robel, 2013; de Groot and Sontheimer, 2011; Yuen et al., 2012). Interestingly, Nakae et al. found that while higher glutamate concentration was in IDH-wt glioma relative to IDH-mt, glutamate was not associated with high preoperative seizure frequency (Nakae et al., 2021). Rather, levels of total N-acetyl-L-aspartate were found to be statistically correlated and were independent predictors of preoperative seizures (Nakae et al., 2021).

Glutamate can be derived from glutamine, the most abundant amino acid in the body. Glutamine transports nitrogen for purine, pyrimidine, and fatty acid biosynthesis and can serve as an agent to fuel the TCA cycle. Conversely, glutamine can be generated from glutamate by a via the glutamine-glutamate cycle (Bak et al., 2006). In summary, glutamate is transported to astrocytes where it is converted to glutamine by glutamine synthetase. The newly synthesized glutamine then arrives at presynaptic neurons where it is hydrolyzed to glutamate by glutaminase, packaged into synaptic vesicles and released into the synaptic cleft during neurotransmission. The phrase “glutamine addiction” is used to describe cancer cells’ exorbitant consumption of glutamine as an energy source and it has been posited that glutamine may be a key marker for glioma progression (Ekici et al., 2020; Márquez et al., 2017; Wise and Thompson, 2010).

Additional Metabolites

Advancements in metabolomics have directed attention at other metabolites and LMWMs that can serve as glioma biomarkers and therapeutic targets, e.g. proline, arginine, methionine, kynurenate, and tryptophan. Proline is oxidized to glutamate in the mitochondria and has been linked to glutamate metabolism in GBM and cell proliferation (Cappelletti et al., 2018). In a recent systematic review, Sawicka et al. found proline to be a prognostic factor and a signal of malignancy (Sawicka et al., 2022). There are also GBM studies that indicate theprognostic potential of arginine, methionine, kynurenate, and kynurenine (Palanichamy et al., 2016; Shen et al., 2018). Kynurenine is a metabolite of tryptophan and is involved in immune evasion in GBM and thus tumor progression and poor prognosis. Overall, metabolomic studies have illuminated how metabolites show promise to be reliable prognosticators (Erb et al., 2008).

Lipids

Though lipidomics is an individual area of study dedicated to identifying and understanding cellular lipids and their pathways, it is worthwhile to discuss key alterations that overlap with metabolomics. Lipids serve as significant infrastructure components across cells and energy sources. For various cancer types, critical lipid metabolism regulators including sterol regulatory element-binding proteins (SREBPs), acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase are up-regulated and extensively covered in another review (Cheng et al., 2018). In glioblastomas, key lipid components that induce cancerous growth and spread include SREBP, sterol-Oacyltransferase (SOAT) and lipid droplets (LDs) (Geng et al., 2016; Yuan et al., 2021). SOAT converts endoplasmic reticulum cholesterol to cholesterol esters (CE) and sequesters CE into LDs to prevent over accumulation of cholesterol and lipotoxicity. Inhibition of SOAT in xenograft models has been shown to inhibit SREBP and thereby reduce GBM growth (Geng et al., 2016). A separate study with a focus on LDs has shown their role in lipid metabolism exploitation by glial and GBM cells (Yuan et al., 2021). Not only has lipogenesis been underscored as a method of GBM progression, but acyl carnitines presence in GBM suggests upregulation of fatty oxidation for GB cellular respiration and proliferation (Lin et al., 2017). In IDH1-mt gliomas, lower levels of fatty acyl chains, triglycerides and sphingolipids are posited to be due to low acyl-CoA synthetase which has conferred better survival outcomes (Zhou et al., 2019).

Treatment

Standard of care treatment for gliomas typically consists of surgical resection and adjuvant chemotherapy and radiotherapy (Stupp et al., 2005), which can lead to treatment-related glioma hypermutation. However, hypermutation has been found highest in early tumorigenesis (Barthel et al., 2019). Recurrent IDH-wt GBM samples from patients who had received chemoradiotherapy and temozolomide (TMZ) were more likely to contain DNA methylation genes, mutations in DNA damage response genes and hypermutated phenotypes due to mutations in mismatch repair genes (Draaisma et al., 2020). These findings further emphasize the necessity of targeted therapeutic approaches for gliomas.

Experimental therapeutic interventions for GBM may involve a combinatorial approach. One such experimental combination approach is fluoxetine (Prozac) and TMZ. TMZ is an alkylating agent which has long been used as a chemotherapeutic agent to treat high grade gliomas due to its ability to methylate guanine at the O6 position which reduces DNA repair capabilities and ultimately induces cell apoptosis (Friedman et al., 2000). Fluoxetine is a SSRI antidepressant capable of selectively
inhibiting sphingomyelin phosphodiesterase 1 (SMPD1) and, when combined with TMZ, is reported to reduce tumors in murine models and increase survival in GBM patients (Bi et al., 2021).

Immunotherapy has emerged as an attractive treatment route, but its challenges are mired in the complexities of brain tumor metabolism in the tumor microenvironment (TME) (Guerra et al., 2020) and CNS anatomy (Engelhardt et al., 2017). Current immunotherapy trials exploring immune checkpoint inhibitors, vaccine therapies, viral introduction to various CNS neoplastic cell lines, and multimodal approaches have had mixed results (Mende et al., 2021).

Overexpression of the G-protein coupled receptor CCR5 across multiple cancer types including GBM has become a niche of neurooncological studies that examine therapeutic targets of this receptor (Lah Turnšek et al., 2021). CCR5 has been implicated in GBM stem cell genesis, invasion, and spread. Kranjac et al. offers an extensive review of the various mechanisms and components at play between CCR5 and one of its ligands, chemokine ligand 5 (CCL5) in GBM (Kranjac et al., 2019). Selective HK2 inhibition has been interrogated as another metabolic target for GBM treatment (Agnihotri et al., 2019). In-vivo chemoradiotherapy studies have shown HK knock out as a promising target (Vartanian et al., 2016) and antifungals ketoconazole and posaconazole have exhibited reduced tumor metabolism and expansion in-vitro, offering evidence for their consideration in clinical trials (Agnihotri et al., 2019).

Natural products derived from vegetables, fruits, spices, and herbs have also been a source of interest for GBM as they are capable of influencing GBM cell cycles, apoptosis, angiogenesis, epigenetic alterations, and reducing therapeutic resistance (Abbas et al., 2020). Flavonoids are naturally present in vegetables and fruits, and the flavonoids apigenin, epigallocatechin, and genistein were observed to selectively activate apoptosis in GBM cells (Das et al., 2010). Quercetin, another extensively studied natural flavonoid in vegetables and fruits including broccoli, red onions, apples, red grapes, cherries, and berries has been found to induce apoptosis in GBM cells and increase chemoradiotherapy effects (Kim et al., 2021; Pozsgai et al., 2013). Amongst polyphenols, resveratrol, a natural polyphenol in mulberries, grapes, and peanuts, increased apoptosis and reversed TMZ resistance in T98G glioblastoma cells, suggesting that certain natural products can serve as a beneficial adjunctive therapy (Huang et al., 2012). A recent review by Abbas et al. covers their molecular mechanisms in greater detail (Abbas et al., 2020).

Resistance
While targeted therapeutics approaches offer benefit to cancer patients outside the CNS based on their tumor’s molecular characteristics, over two decades of data demonstrate that this overall approach suffers from several weaknesses in high grade glioma treatment. Most prominent among these is the propensity of subpopulations of heterogeneous tumor cells to subvert drugs that act via apoptosis, by far the most prevalent cell death mechanism engaged by currently employed therapeutic agents, leading to therapeutic resistance and poor patient outcome. Two models theorize cancer cell heterogeneity and its consequences: clonal evolution and the cancer stem cell (CSC) hypothesis. The clonal evolution theory, proposed by Peter Nowell in 1976, suggests that the accumulation of cellular mutations via heritable and epigenetic changes generate clonal outgrowths that thrive in response to microenvironmental selection pressures (Nowell, 1976). In effect, the model resembles the Darwinian model of evolution. The application of this theory in GBM cells has been observed in a study by Wang et al., who found increased mutation in recurrent GBM that had been treated with standard therapy including TMZ, indicating that resistant clones survive and hypermutate when confronted with drug-induced pressure (Yuzawa et al., 2016).

The cancer stem cell (CSC) hypothesis first manifested in brain tumors in 2004, when Singh et al. found a subset of GBM stem cells (GSC) that were capable of initiating tumor growth via CD133+ protein surface markers (Singh et al., 2004). GSCs have since been observed to possess chemoresistance (Eramo et al., 2006) and human mesenchymal stem cells (hMSCs) inside glioma-associated hMSCs have been linked to dismal outcomes (Shahat et al., 2017). Chemoresistance is in part due to the increased expression of tumor growth factors such as VEGF and stromal derived factor 1 (SDF-1) which promote angiogenesis and appear to provide resistance against standard therapies such as radiation (Folkins et al., 2009; Harmey and Bouchier-Hayes, 2002). CSCs are also able to prevent cytotoxicity through high drug efflux by ATP-binding cassette (ABC) transporters, which are ATP-dependent membrane-bound proteins (Vasiliiou et al., 2009). Overexpression of ABCB1, ABCB5, and ABCA13 (three ABC transporters) were found to increase TMZ resistance (Chou et al., 2012; Dréan et al., 2018; Lee et al., 2020).

In addition to clonal evolution and stem cell capacity to shape and evade cell death with TMZ, another response from GBM cells upon TMZ administration is over expression of the DNA repair protein O-6-methylguanine-DNA methyltransferase (MGMT), which confers resistance and shorter survival (Perry et al., 2017). Therapeutic resistance has also been linked to microRNA (miRNA) dysregulation. Shi et al. found that U87MG GBM cells developed resistance to TMZ by upregulation of miRNA-21 which decreased Bax/Bcl-2 and caspase-3 activity (Shi et al., 2010).

Conclusion
Despite scientific advancements made in cancer research, high grade gliomas remain enigmatic. Just as the discovery of IDH mutation sparked great interest in cancer metabolism in the neuro-oncology field, we have the opportunity to take it to the next step using metabolomics to help understand disease progression and changes in cancer profiles across all cancer types (Crooks et al., 2021; Kwon et al., 2015). Our summary of metabolomic studies that pertain to high grade gliomas, spanning metabolic pathways, metabolites, and therapeutic interventions underscores the significance of metabolomics as a way to better understand and treat high grade gliomas.

Acknowledgments
The authors thank Dr. Robert O’Donnell for his expert review. Dr. Aboud is supported in part by the UC Davis Paul Calabresi Career Development Award for Clinical Oncology as funded by the National Cancer Institute/National Institutes of Health through grant #2K12CA138464-11.


