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REVIEW ARTICLE

Bioengineered Models to Study Microenvironmental Regulation of Glioblastoma Metabolism

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Abstract

Despite extensive research and aggressive therapies, glioblastoma (GBM) remains a central nervous system malignancy with poor prognosis. The varied histopathology of GBM suggests a landscape of differing microenvironments and clonal expansions, which may influence metabolism, driving tumor progression. Indeed, GBM metabolic plasticity in response to differing nutrient supply within these microenvironments has emerged as a key driver of aggressiveness. Additionally, emergent biophysical and biochemical interactions in the tumor microenvironment (TME) are offering new perspectives on GBM metabolism. Perivascular and hypoxic niches exert crucial roles in tumor maintenance and progression, facilitating metabolic relationships between stromal and tumor cells. Alterations in extracellular matrix and its biophysical characteristics, such as rigidity and topography, regulate GBM metabolism through mechanotransductive mechanisms. This review highlights insights gained from deployment of bioengineering models, including engineered cell culture and mathematical models, to study the microenvironmental regulation of GBM metabolism. Bioengineered approaches building upon histopathology measurements may uncover potential therapeutic strategies that target both TME-dependent mechanotransductive and biomolecular drivers of metabolism to tackle this challenging disease. Longer term, a concerted effort integrating in vitro and in silico models predictive of patient therapy response may offer a powerful advance toward tailoring of treatment to patient-specific GBM characteristics.

Key Words: Bioengineered platforms, Biomimetics, Cancer metabolism, Glioblastoma, Mathematical modeling, Microphysiological platforms, Tumor microenvironment.

INTRODUCTION

Glioblastoma (GBM) is the most common malignant primary brain tumor, with one of the worst prognoses of all human cancers (1). It accounts for 20% of all intracranial tumors and 52% of parenchymal brain tumors and is classified as a grade IV astrocytoma by the World Health Organization (2). GBM is diagnosed by histopathology of a brain tumor biopsy when the pattern of growth shows high mitotic rate, cellular and nuclear pleiomorphisms, microvascular proliferation, necrosis, and pseudopalisading of tumor cells. Many of these histologic features suggest a landscape of differing oxygen and nutrient availability in a background of high metabolic demand of rapid mitosis and cell mobility. Only minor improvement in overall survival has been demonstrated in over 30 years (3), although some newer molecular details are being discovered at a rapid pace. Current clinical approaches are standardized to the Stupp protocol: combination therapy including maximum safe resection, external beam radiation, and chemotherapy with temozolomide (4). However, even with extensive interventions, median life expectancy is only 12–14 months (5).

Genomic, transcriptomic, and proteomic tools have unveiled critical genes, proteins, and signaling pathways dysregulated in cancer (6). Large-scale genomic analyses, such as The Cancer Genome Atlas, have uncovered core pathways involved in regulation of GBM proliferation, invasiveness, and DNA repair, as well as somatic landscapes with unprecedented resolution revealing immense inter-tumor complexity and heterogeneity (7). Transcriptional profiling indicates a spectrum between proneural and mesenchymal subtypes, which correlates with disease aggressiveness and prognosis (8); however, transcriptomic classification has struggled to predict survival and therapeutic vulnerability. It has been challenging to apply

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data gleaned from omic studies as they may not distinguish driver from passenger mutations, making identification of critical pathways challenging. The challenge of identifying key molecular targets from the immense list of aberrantly expressed genes, as well as the failure of genomic and transcriptomic tools to respectively account for epigenetic and post-translational modifications are important hurdles in the understanding of GBM biology (9).

The emerging field of metabolomics aims to complement the existing omics studies by providing functional, global assessments of patients, taking into account the genetic alterations, activity of enzymes, and changes in metabolic reactions; metabolomics provides the functional downstream readouts of the genomic, proteomic, and transcriptomic upstream alterations (10–13). Although metabolomic investigations will inevitably generate large sets of important findings, its utility, particularly with respect to clinical gains, relies on the mechanistic understanding of GBM metabolism, which currently remains limited. From the documentation of metabolic dysregulation in cancer via the Warburg effect to the varied routes of cancer metabolism via nucleotide, lipids, and proteins, much progress has been made to decipher how cancer evolves to fulfill its energy requirements. However, more recent reports have shed light on the surprising impact of external cues, specifically those embedded in the tumor microenvironmental (TME), that can dramatically reshape the metabolic profile of cancers. These studies represent the next wave of metabolism focused studies, but technical challenges and the lack of experimental tools represent obstacles to deeper mechanistic examinations. New approaches based on understanding of GBM biology, including metabolic and TME influences, are needed.

In this review, we focus our discussion on the progress of bioengineering tools (in vitro and in silico) and approaches that enable robust interrogation of GBM metabolism. We touch briefly on the features of the TME that influence GBM metabolism—there are many excellent and thorough reviews on this topic (14, 15). Lastly, we describe current therapeutic strategies that utilize metabolic targeting and offer perspectives on the future integration of bioengineering models for GBM metabolism studies.

DYSREGULATION OF GBM METABOLISM

Despite displaying genomic and transcriptomic heterogeneity, most cancer cells, including GBM, exhibit characteristic perturbations in metabolism. Classically, the Warburg effect has been observed in cancer, where cells choose an energetically unfavorable route relying on aerobic glycolysis even in the presence of oxygen (16). Although this has been broadly applied to cancers, a more nuanced picture can be seen in various types of cancers. For example, GBM cells have been largely unaffected by treatment with glycolysis inhibitors, suggesting that more substrates are metabolized to support the energy demands of the cells (17). In fact, more recent work has revealed that GBM utilizes bioenergetic substrates such as amino acids, nucleic acids, and fatty acids, with emerging evidence that fatty acid metabolism is the primary substrate for energy production (18, 19). The collective metabolic process of GBM ultimately leads to an acidic environment that is harmful to normal cells but has minimal effect on cancer cells, thus supporting tumor progression (20).

Much progress has been made in documenting the various pathways that cancer cells can take to fulfill their biosynthetic needs; however, it is increasingly apparent that these processes are heterogenous, adaptable, and subject to external cues. The dynamic TME presents challenging conditions that confer metabolic stress and nutrient deprivation to tumor cells, forcing them to shift their metabolic pathways to survive. For example, glioblastoma stem cells (GSCs) in perivascular regions exhibit robust glycolytic metabolism based on blood glucose availability, whereas cells in hypoxic regions display high levels of metabolic flexibility and are fueled by lactate, lipids, and amino acids (21). Further, mechanical changes in the GBM TME, such as extracellular matrix (ECM) stiffening, and remodeling, results in an accumulation of glutamate and dysregulated TCA cycle via the YAP/TAZ axis (22). The interrogation of TME dependent metabolic changes represents a new frontier in GBM metabolism research.

MICROENVIRONMENTAL REGULATORS OF GBM METABOLISM

The TME also encompasses dynamic array of biochemical and biophysical signals that synergizes to influence GBM progression and metabolic plasticity (Fig. 1). The marked regional variability within GBM architecture lends itself to unique cellular, metabolic, anatomical, and biophysical environments. Pseudopalisading tumor cells surround a hypoxic, necrotic core, while other areas are supplied by leaky, tortuous neovessels in response to aberrant angiogenesis. The ECM is varied in composition and physical characteristics throughout, populated by stromal cells such as tumor-associated lymphocytes and macrophages that secrete growth factors and cytokines to produce a uniquely altered milieu (24). Vascular pericytes and endothelial cells, which are induced to proliferate by GBM growth factors may synergistically drive tumor growth but are not themselves malignant (25, 26). The inherent TME heterogeneity adds layers of complexity to how metabolism may be regulated (27).

GBM Cell-Stroma Interactions in TME

The outdated term, glioblastoma multiforme, underlines the heterogeneous composition of the tumor and its irregular architecture. GBM consists of differentiated bulk tumor cells, stromal cells, and multiple populations of GSCs, a privileged population of tumorigenic stem cells that are capable of self-renewal and asymmetric differentiation into bulk tumor cells (28, 29). A heterogeneous GBM cell population coupled with epithelial to mesenchymal transitions may account for different GBM subtypes (30), ranging from proneural to mesenchymal (8), with the latter being the most aggressive and refractory to treatment, possibly due to a high population of GSCs within these tumors (31), and with metabolic profiles being subtype-dependent (21). Fast-cycling and slow-cycling cells in GBM, respectively, reflecting relative utilization of glycolysis and oxidative phosphorylation, have been identified

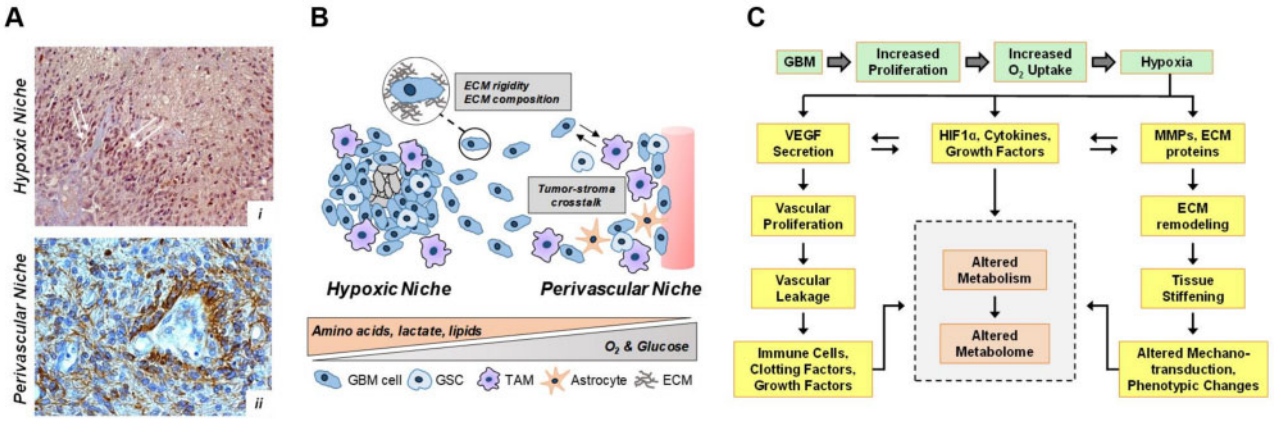

FIGURE 1. GBM metabolism is influenced by cues within the TME. **(A)** Hypoxic and perivascular niches possess unique characteristics with (i) hypoxic niches activating HIF1α (arrows) around perinecrotic regions and (ii) perivascular niches integrating vascular, stromal, and tumor components. Reprinted with permission from (23). **(B)** GBM cells and stroma cells compete for and share metabolites within the TME to shape tumor metabolism along with cues from ECM composition and rigidity. **(C)** Key TME conditions can be derived from these complex interactions and potentially evaluated under controlled conditions in bioengineering models.

(32). Interestingly, GSCs proliferate at a lower rate and have a different metabolic profile compared to bulk tumor cells (33). These metabolic differences may be attributed to mitochondrial fragmentation, a feature more commonly observed in GSCs (34). Alterations in mitochondrial dynamics and fusion alter the balance of metabolism and are a rapidly progressing area of study (34–36).

In addition to these intrinsic differences between bulk tumor and GSCs, the TME presents significant interplay between stromal and tumor cell metabolism. With the high energetic demands of GBM cells, nutrients like glucose are quickly metabolized, leading to competitive uptake between tumor and stromal cells that shapes the TME toward a pro-tumorigenic state. For example, immune cells, such as T cells, natural killer cells, and neutrophils, all arrive within the GBM TME; however, the rapid uptake of nutrients along with poor vascularization within the TME prevents immune cells from robust activation and cytotoxic functionality (37). Additionally, myeloid-derived suppressor cells metabolize amino acids that maintain T-cell activity and further promotes the production of reactive oxygen species that suppress the anti-tumor effect of the immune cells (38). Tumor-associated immune cells, including various lymphocyte types and macrophages, display either suppressing or stimulating signals to T cells and may interfere with normal immune surveillance. The competitive interplay of tumor and stromal cells is thought to be responsible for GBM resistance to immunotherapy (39).

Other populations of cells in the GBM TME create a symbiotic relationship with the tumor cells to support disease progression and evolution. The surrounding endothelium has been shown to secrete catabolites, such as pyruvate, lactate, glutamate, and alanine to support GBM metabolism (40). More differentiated tumor cells in normoxic conditions have been shown to feed metabolites toward tumor stem cells in hypoxic conditions (41). Transportation of nutrient cargoes via extracellular vesicles containing fatty acids, amino acids, and TCA metabolites to tumor cells have also been observed by cancer associated fibroblasts and mesenchymal stem cells (42). The intimate interplay between non-neoplastic cells and the tumor population clearly reveals a complicated metabolic interconnectivity that remains poorly understood.

Metabolic Differences Between Hypoxic and Perivascular Tumor Regions

Two of the most studied regions of the GBM TME, the perivascular and hypoxic niches, play crucial roles in tumor maintenance and progression (43). While GBM cells generally display aerobic glycolysis, tumor cells in perivascular tissue defy the Warburg effect in favor of oxidative phosphorylation and are intensely anabolic (44). In contrast, hypoxic tumor tissue undergoes its own set of changes which are largely mediated by hypoxia-inducible factor 1α (HIF-1α).

Hypoxia, an established factor that regulates GBM survival and chemoresistance (45), is most obviously linked to tumor metabolism. Hypoxia-inducible factors (HIF) transcription factors represent the master regulators of the hypoxic response and are tightly controlled by the enzymatic function of prolyl hydroxylases, which are 2-oxoglutarate and iron-dependent dioxygenases that function to destabilize HIF in normoxic conditions (46). However, low oxygen tension inhibits the oxygen-dependent prolyl hydroxylases, leading to the stabilization and accumulation of HIF transcription factors. HIF-1α mediates multiple cellular adaptations present in GBM under hypoxic conditions, such as cell differentiation, inflammatory/immune response modulation, and metabolic reprogramming (47, 48). It also promotes signaling pathways involved in cell survival, adenosine-mediated chemoresistance, and mitochondrial NIX-mediated mitophagy (49). HIF-1α has been shown to influence metabolic profile in a variety of ways. Through Ras-mediated HIF-1α signaling, GBM cells display reduced mitochondrial respiration and subsequent aerobic glycolysis. HIF-1α can also act through the TP53-

induced glycolysis and apoptosis regulator (TIGAR) to control glycolysis in GBM. Further, HIF-1α has recently been shown to regulate branched-chain amino acid (BCAA) transporters to affect BCAA metabolism (leucine, isoleucine, and valine) through binding to the hypoxic response element of the *BCAT1* gene (50). Accordingly, inhibition of HIF-1α related pathways has been shown to dramatically alter metabolism. Disruption of the HIF-1α-PDK1 axis has led to a stark reduction in glycolysis and transition to oxidative phosphorylation, signaling a shift from Warburg metabolism (51).

Notably, low tissue pH and lactic acidosis, a hallmark of GBM that is intimately tied to hypoxia and glycolysis, impacts tumor cell metabolism and survival. Extracellular acidic environments increased surface cholesterol expression in LN229 GBM cell lines, and cholesterol depletion of cells adapted to acidic pH decreased cell survival (52). Specifically, in GSCs, the scavenger receptor CD36 binds to oxidized low-density lipoprotein, which maintains the GSC population through increases in proliferation (53). De-acidifying endolysosomes in GBM cells allow LDL cholesterol accumulation, thus reducing cell viability and proliferation potential (54). cAMP activators, such as cAMP/CREB/PGC-1α regulate lactic acid levels and dependency on oxidative phosphorylation to regulate TME pH (55, 56).

In contrast to cells in the hypoxic regions, cells in perivascular tumor regions are presented with ample amounts of oxygen, glucose, and other nutrients. Within the perivascular niche, one significant change is the activity to Notch signaling that controls metabolic adaptations of tumor cells. Perivascular GSCs display increased levels of Notch signaling not seen in hypoxic regions that is sufficient for the suppression of glycolysis (57), particularly in CD133 expressing cells. Similarly, CBF1, a regulator of Notch signaling involved in epithelial to mesenchymal transitions and formation of GSCs, also reduces glycolysis (58). Perivascular regions also have much higher glutamine and glycine content than hypoxic regions, demonstrating differential amino acid metabolism according to proximity to oxygen from blood vessels (59). In addition to these region-specific changes, a metabolic interplay has been posited in the perivascular and hypoxic regions where lactate release from hypoxic regions are metabolized through oxidative phosphorylation by perivascular cells and glucose release from the perivascular cells fuel glycolysis in the hypoxic space (60). These findings collectively demonstrate the niche-specific metabolic control but also dynamic relationship that exist to drive metabolic changes.

Altered ECM Regulates GBM Metabolism Through Molecular Interactions

The GBM ECM displays a wide array of aberrantly expressed ligands and signaling molecules that modulate tumor cell proliferation, invasiveness, and aggressiveness. Elevated hyaluronic acid (HA) increases tumor stiffness, and higher levels of CSPGs, such as brevican and versican, promote invasiveness, and proliferation (61). Tenascin-C (TNC) and tenascin-R also promote angiogenesis, proliferation, and invasiveness (62). Notably, TNC secretion is regulated by HIF-1α leading to tissue stiffening and subsequent activation of a positive feedback loop wherein increased rigidity leads to further HIF-1α-dependent TNC secretion (63). Laminin, a component of basement membranes, is functionally linked to Notch signaling and likely to regulate tumor metabolism through its control of glycolysis. Other ECM components that are up-regulated in malignant gliomas include SPARC, hevin, testicans, fibulin, and heparan sulfate proteoglycans. In the midst of these varied ECM signals, evidence shows that metabolic adaptability occurs primarily through transduced signals from focal adhesions. The activation of PI3K signaling downstream of focal adhesions leads to an increase in glycolytic activity with additional reports connecting PI3K signaling and glucose transporters, which increase the glucose flux within the cell (64, 65). HA receptor HMMR was also recently linked to glycolytic control (66). In an unbiased analysis of gene expression and glycolytic phenotype, HMMR was the second most correlated gene in a panel of breast cancer lines. Interestingly, HA degrading enzymes were also shown to increase glycolysis in a panel of cultured cells, including GBM U87s (66).

ECM composition is not homogenous or static, in fact, ECM composition varies between different TME regions; periostin and MMP-2/9 are expressed in hypoxic regions (67), whereas type I collagen, tenascin C, laminin, integrin-α6, and fibronectin are more abundant in vascularized regions (Table). ECM composition is also related to GBM cell phenotype. Up-regulation of quiescent GBM cells was identified with up-regulation of laminin, collagens, tenascin C, and integrin α3 (62). Other studies have demonstrated flexible pro-invasive ECM remodeling in the mesenchymal subtype, establishing a feed-forward loop for tumor remodeling (69). Conversely, physical compaction due to proliferating GBM cells induces collagen types IV and VI expression (70). These studies highlight the complex relationship between ECM composition and tumor metabolism and present mechanisms that are still being elucidated.

Biophysical Cues Modulate GBM Metabolism

Although mechanical changes in GBM tumors have been well documented, more recent reports have uncovered a more nuanced perspective regarding tissue rigidity. Enhanced stiffness has been observed via ultrasound elastography; however, softer tumor profiles and mechanical heterogeneity have also been demonstrated, revealing the need for more robust biophysical characterization with higher resolution so that various regions within the TME can be tested (71). Given the dynamic nature of the TME, biophysical changes likely occur during tumor evolution and locally instruct tumor cell phenotype and function. Nonetheless, the effect of biophysical TME characteristics on GBM tumor cell biology such as survival, proliferation, and invasiveness is well-established with emerging trends coming into focus on metabolism control (72, 73).

Increased HA production in GBM is a major contributor to TME stiffness, and is associated with a nonspecific increase in metabolic activity (74). HA production is also associated with hypoxic TME conditions and increased glycolysis (75). Interestingly, HIF-1α induces procollagen-lysine, 2-oxoglutarate 5-dioxygenase (PLOD) expression, which then increases collagen crosslinking and thus tumor rigidity (76). Meanwhile,

TABLE. ECM Abundance in Hypoxic and Perivascular Regions

ECM Composition	Hypoxic Region	Perivascular Region
Hyaluronic Acid	++	+
Periostin	++	−
Tenascin C	+	++
Collagens	−	++
Laminins	−	+++
Fibronectin	−	++

GBM ECM composition varies between hypoxic and perivascular niches. Signs qualitatively indicate presence (+) or absence (−) of typical elements found in GBM ECM (68).

IDH1-mutant tumors can exhibit decreased stiffness, due in part to inhibition of HIF1α-TNC signaling (63). Furthermore, GSCs and differentiated tumor cells exhibit differing metabolic profiles in soft versus stiff microenvironments. Stiffened microenvironments can activate the PI3K/Akt pathway, which represent a mechanotransductive link to increased glycolysis (77). Recent work has also shown the impact of mechanosensation, differentiation, and metabolism in GBM. Hughes et al (78) showed that the mesenchymal growth factor, BMP4 promotes GSC differentiation and leads to reduced oxidative phosphorylation and differential cell spreading on soft and stiff substrates. Inhibition of oxidative phosphorylation surprisingly disrupts cell protrusive extensions and spread area, underscoring a complex interaction between GBM metabolism, cell mechanotransduction, and TME biophysical characteristics (78).

BIOENGINEERING MODELS ENABLE DEEPER INTERROGATION OF GBM METABOLISM

Mechanistic understanding of cancer metabolism has largely been dependent on available experimental tools. Conventional in vitro assays have historically utilized monotypic cell populations cultured on plastic substrates, which fail to include important aspects of in vivo tumor growth, such as tissue architecture/composition, vascularization, and 3D cell-cell interactions (79). As insights regarding the impact of the TME on metabolic regulation become clearer, it is apparent that a new suite of experimental platforms is needed to recapitulate these critical cues and more accurately describe mechanisms of metabolic control. Emerging bioengineering tools include 3D cell culture systems and mathematical modeling, designed to simulate evolving TME conditions.

3D Cell Culture Models

Spheroid-Based Models

As scaffold and hydrogel-based 3D cell culture models have improved, evidence of their ability to more accurately model in vivo cell behaviors have become readily accepted, with studies targeting cell morphology, proliferation, differentiation, invasion, and metabolism (80). 3D culture models can preserve cell-cell, cell-matrix, and ECM components in a more physiologically relevant context, and although challenges exist in controlling these interactions, these models have been valuable in recapitulating TME cues for metabolic studies. Within GBM, Ma and colleagues utilized 2D and 3D Poly-lactic acid scaffolds to compare the molecular changes via DNA microarrays (81). When cultured in 3D, GBM cells exhibited major enrichments in key transcription factors related to lipid metabolism and hypoxia when compared to 2D culture under the same conditions. Ca-Alginate scaffolds have similarly been used to compare GBM cells in 2D and 3D, revealing increases in pathways regulating fatty acid and nucleotide metabolism (82). This report also showed alterations in drug metabolism with 3D cultured spheres exhibiting increased drug resistance through upregulation of cytochrome P450 related genes, which function as intracellular drug inactivation enzymes.

In addition to the physical 3D environment, ECM composition has been shown to greatly influence cellular behavior and metabolic activity. Unlike the ECM of other solid tissues, brain ECM is enriched in glycoproteins, such as tenascin and link proteins, glycosaminoglycans, such as hyaluronic acid (HA), and proteoglycans, such as aggrecan, neurocan, versican, and phosphacan (83). Fibrillar ECM proteins like fibronectin and collagen are sparse in comparison to other tissues. HA, a polyanionic glycosaminoglycan, in particular, has become intensely studied as it is the most abundant brain ECM protein and plays important roles in normal brain maintenance and pathological processes (75). HA-based platforms have yielded crucial insights toward the role of HA in facilitating GBM proliferation, invasion, and angiogenesis (72). Recent work has begun to uncover the role of HA in regulating metabolic activity. Using high resolution 2-photon metabolic imaging, quantitative readouts of metabolic perturbations were observed in HA-infused bioengineered tissue models (84). These spheroid-based models demonstrated significant interplay between spheroid edges and HA altering not only cell morphology of the cells but also metabolic profiles through enriched glycolysis and fatty acid oxidation and synthesis (84). HA of different molecular weights (10, 60, and 500 kDa) also influence metabolic activity of GBM cells (75). Matrix bound HA of all molecular weights increased overall metabolic activity when compared to non-HA controls; however, 60 kDa HA had the greatest effect on enhancing overall metabolism. Notably, the functional role of CD44, the main receptor for HA, has been implicated in cancer metabolism with CD44 knockdown decreasing glucose uptake, ATP production, and lactate production (85).

Three-dimensional models represent an advance over 2D platforms as they include contributions from cell-cell, cell-matrix, and ECM contributions; however, it is challenging to control the magnitude of these cues in these spheroid-based models and additionally integrate multicellular populations. These studies highlight the broad impact of the 3D microenvironment and show its utility as a drug screening tool.

Organoid Models

Tumor organoids represent a growing effort to more accurately model the 3D cancer TME through the generation of

multicellular cultures that mimic tumor heterogeneity and architecture. Organoids are developed through the expansion of tissue explants from patients, which are then maintained under specific non-adherent culture conditions. Organoids self-organize and are capable of recapitulating genetic, microenvironmental, and histopathological characteristics of original tumors (86). Additionally, these models can be derived from various genetic and subtype specific backgrounds and preserve interactions between tumor, immune, and stem cells. Although this technology is still immature and without a clear standardized protocol in GBM, early reports reveal exciting directions for GBM metabolism studies (87, 88). Emergent GBM organoids can recapitulate tumor heterogeneity as well as hypoxic gradients (Fig. 2A). Further, GBM organoid models can also mimic transition zones between nutrient-rich and nutrient-poor regions (88). The ability to model these characteristic features of GBM presents a more accurate representation of the metabolic profiles associated with tumor heterogeneity and thus show its utility as a drug screening tool. Early reports reveal that GBM organoids are able to recapitulate the clinical response to standard temozolomide treatment and also mirror drug responsiveness in MGMT methylated and unmethylated samples. Examination of a panel of drugs reveal that 2D platforms are much more sensitive to treatment when compared with organoid systems due to their monotypic nature and their lack of multicellular populations. In addition to testing drug efficacy, GBM organoids have offered insights regarding metabolic differences in various tumor regions. Metabolic examination of GBM organoids has revealed clear zones of altered metabolism with lipid metabolism being highest in hypoxic and perinecrotic regions (88). These metabolic changes can be probed through RNA sequencing at specific organoid sites, or by metabolic imaging via multiphoton microscopy through redox ratios of the fluorescence lifetime of NAD(P)H and FAD (86).

GBM organoid models enable the self-organization of patient-derived tissue and provide the integration of the multicellular compartment of GBM tumors. Through the genetic and subtype-specific propagation of GBM tumors, these systems provide a strong clinically relevant tool for drug metabolism studies and basic science examination of TME control of metabolism.

Microphysiological Systems

Recent advances in engineered platforms have led to the creation of more sophisticated systems that incorporate multicellular components along with architectural control to describe various aspects of cancer niches within the TME. These platforms specialize in the isolation of factors for study, incorporating various cell-cell and cell-ECM interactions in controlled spatial organization and under various flow conditions. These systems offer the advantage of probing specific multicellular interactions under physiologically relevant settings. Coculture microfluidic platforms for modeling the GBM perivascular niche have revealed important interactions that exist between endothelial cells and GBM cells in regulating tumorigenicity (89). Within these systems, GSCs exhibited pro-invasive genes, CSC stemness markers, and CXCR4 signaling, and exhibited invasive behaviors reminiscent to those observed in vivo. However, in GBM these platforms have mostly been used to study drug efficacy, immunosuppression, and angiogenesis, with few directly focused on the role of the GBM TME on GBM metabolism. We briefly detail innovations in microfabrication techniques that enable simulation of biophysical and biochemical cues within the TME of other cancer types and may also hold potential in the study of TME regulation of GBM metabolism. Ayuso et al created a tumor-on-a-chip device that enables examination of how cancer metabolism creates "starvation gradients" within the microenvironment that lead to cell proliferation or necrosis (Fig. 2C) (90). Within this system, the device can be disassembled and subjected to gene profiling and sequencing strategies at specific sites, allowing for elucidation of genomic and transcriptomic alterations. A vascular microtumor platform described by Sobrino et al (92) successfully integrated stromal cells, perfused endothelial components, and tumor cells within a 3D ECM (Fig. 2B). The vascular microtumor determined metabolic profile through fluorescent lifetime imaging microscopy that can detect free or protein bound NADH and determines their ratio, which can indicate glycolysis or oxidative phosphorylation. Within this organization, all cell populations showed metabolic heterogeneity with endothelial cells becoming reliant on oxidative phosphorylation when flow was removed. Tumor cells were the most glycolytic, as expected, with stromal cells exhibiting the least glycolytic profile and endothelial cells falling in between. Drug treatment experiments also reveal metabolic changes to treatment, which may represent a mechanism by which GBMs resist therapy. Visualizing the dynamics of tumor metabolism in these controlled environments represent a strong tool for identifying suitable metabolism-focused therapies.

As these systems begin to unravel the complex biophysical, biochemical, and cellular interactions that exist within the TME, they may enable a new generation of investigations that could begin to decouple the relationship between the TME and GBM metabolism. The ongoing pursuit will require an integrated strategy that can incorporate these cues and evaluate their contributions during tumor evolution. In this regard, mathematical modeling may provide an important integrative approach toward metabolic discovery in GBM.

Mathematical Models

An integrated analysis of TME and metabolic data with the goal of incorporating TME-induced metabolic adaptation and with the potential to predict therapy response requires a systems-level approach. In this context, "systems-level" implies an analysis that considers emergent behavior arising from the interaction among individual components. Mathematical and computational analyses are ideally suited for systematic evaluation of tumor parameters. This evaluation through bench-based empirical approaches would be impractical due to time and cost constraints, although high throughput molecular discovery platforms are capable of testing a few parameters at a time over thousands of data points. These analyses can arrive at an unbiased comprehensive analysis, in con-

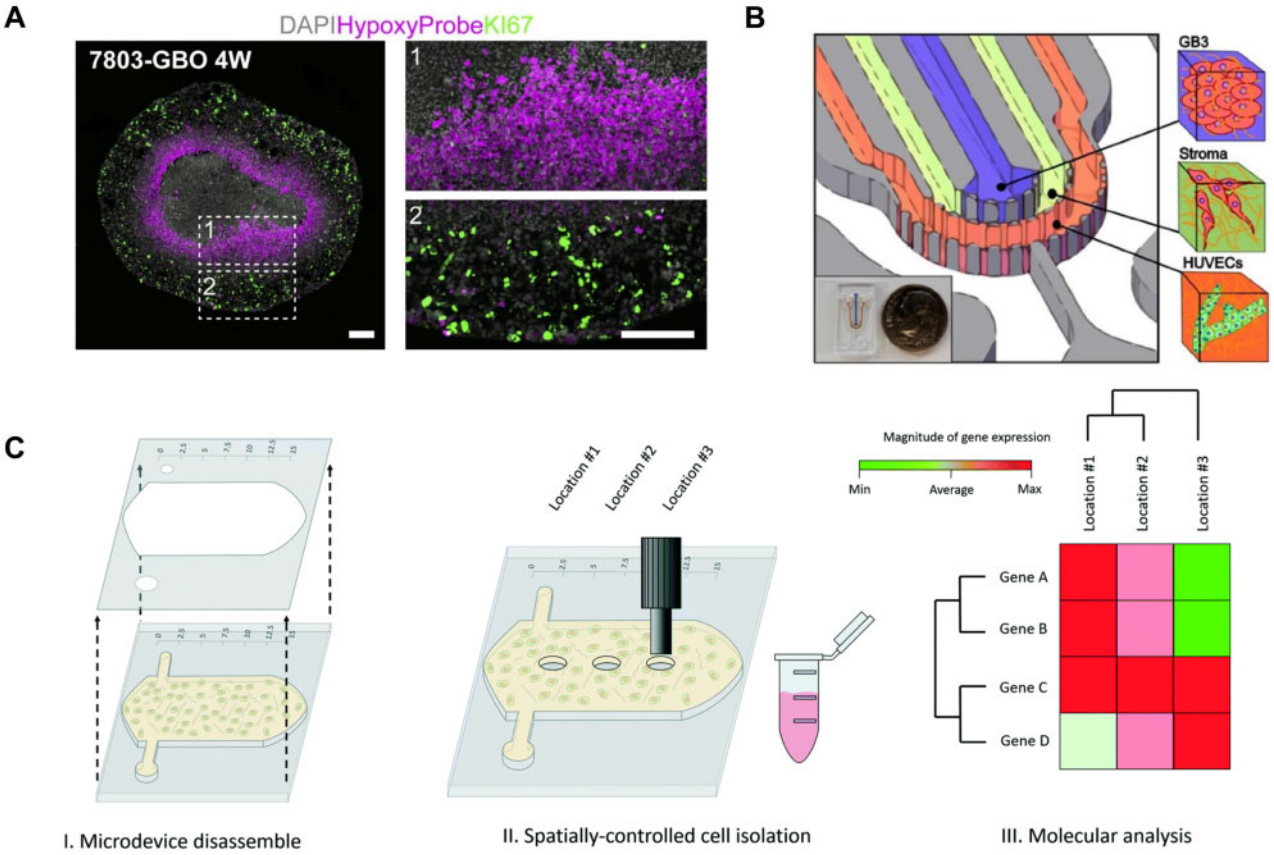

FIGURE 2. Advanced bioengineered culture systems enable molecular and functional analysis of metabolism. Tumor organoids can recapitulate architectural features and hypoxia gradients in GBM **(A)**. Microphysiological systems allow for experimentation with multiple cell populations (GB3—GSC line, Stromal cells, and HUVECs) **(B)** and offer spatially controlled examination of molecular changes **(C)**. Modified and reproduced with permission from (89–91).

trast to candidate gene approaches, e.g. evaluation of PTEN associated with gene mutations. Mathematical modeling has been applied to study glioma progression and treatment response, as well as cancer metabolism, and cancer biomechanics in general, but not necessarily their combination in the context of GBM. In particular, the consideration of TME conditions could provide additional insight into metabolic adaptation to the TME. Metabolome-centered analyses have benefited from network-oriented techniques, such as principal network analysis, as well as data-driven methods, such as machine and statistical modeling (11). The combination of these methods with spatio-temporal representations of tumor growth enables mechanistic evaluation of TME contributions that may unravel new insights to these combinatorial effects.

Analysis of GBM Metabolism

Several studies have evaluated metabolites from adult brain tumors, as recently reviewed (11). In particular, the landscape of metabolic-transcriptional alterations in GBM was evaluated (93). The study applied integrative network modeling, which is especially suited to analyze omic data, to analyze both metabolic and transcriptomic datasets. The model yielded 4 distinct metabolic-transcriptomic signatures capturing: hypoxia, cell-cycle functions, immune response, and oligodendrocytic differentiation. The findings emphasized the association of metabolism dysregulation with oncogenic signaling alterations, including alterations of the cell-cycle. In this manner, the modeling enabled analysis of oncogenic transcriptional alterations resulting from metabolic dysregulation.

Modeling GBM Interactions with the TME

The potential of mathematical modeling to systematically evaluate TME-induced metabolic changes depends crucially on known TME biological knowledge. In this regard, advances in experimental models, such as 3D cell culture platforms are especially suited to offer quantitative measurements that can be linked to mathematical model parameters, particularly based on histopathological evaluation. Densities of tumor and microglial cells, and concentrations of growth factors and other signaling molecules in the GBM TME were simulated via reaction-diffusion equations to capture their interactions (94). The mathematical model was applied to a transwell experimental assay to show that microglia could stimulate tumor cell invasion by secreting TGF-β. The model consistently predicted the role of glioma-infiltrating-macrophages in promoting glioma invasion in vitro, while presence of astrocytes and

MMP inhibitors was shown both theoretically and experimentally to block GBM invasion. The model was thus able to replicate the main experimental findings and offer the capability to explore the development of new therapeutic approaches. Recently, a computational model simulated GBM biomechanics as it grows and invades surrounding tissue (95). Cell proliferation and migration, represented by a reaction-diffusion process, were coupled to the mechanical interaction, representing tissue as a linear elastic material, by linking local increase in tumor cell concentration to tissue isotropic strain. Results showed invasiveness consistent with simulation parameters, and yielded tumor-induced pressures of biologically realistic magnitudes.

The crosstalk between vascular endothelial cells and GSCs has been shown to promote GSC self-renewal and tumor progression (96). GSC can generate vascular pericytes (97) and trans-differentiate into vascular endothelial cells (GEC), which potentially inherit mutations present in GSC. A 3D mathematical model of GBM was employed to study trans-differentiated vascular endothelial cells mediation of resistance to current GBM therapies (98). The model predicted that GSC can drive GBM invasiveness and that GEC can form a network within hypoxic tissue, consistent with experimental observations (99). Simulation of standard-of-care treatments (radiation with temozolomide) together with anti-angiogenic therapies (avastin) decreased tumor size but increased invasiveness. Anti-GEC treatments blocked GEC support of GSC and decreased tumor size but also led to increased invasiveness. Anti-GSC therapies that promote differentiation or disturb the stem cell niche reduced both simulated tumor size and invasiveness (98), but could not completely eradicate it since GSC are maintained by GEC.

Modeling GBM Metabolism and Its Interaction with the TME

The connection between cell metabolism and GBM aggressiveness was explored with a 3D mixture model in Bearer et al (100). Two cell clones, one with lower and one with higher metabolic demand were simulated to drive GBM invasion in a 3D spatio-temporal model of tumor growth, in which tumor morphology was simulated as a function of tissue pressure. As the overall tumor evolved, the more aggressive clone, with higher metabolic demand, first invaded into surrounding, less aggressive, intra-tumoral tissue, and then became dominant in driving overall invasiveness (Fig. 3). In hypovascularized regions, this invasiveness was in the form of palisading cells and slender fingering protrusions, as proliferation decreased and migration was up-regulated due to lack of oxygen and nutrients. In contrast, invasiveness was in the form of budding, broader clusters in more vascularized tissue, as proliferation resumed and migration decreased. These modeling results provide a mechanistic, pressure-based link between shifts in metabolism and corresponding changes in the tissue-scale invasiveness. This modeling approach demonstrated the emergence of new subclones of malignant cells within the tumor mass, highlighting that heterogeneity includes both tumor cells and their environment.

Despite these advances, representation of metabolic activity in these models remains rudimentary. Inclusion of TME conditions that modulate model parameters would enable customization to tumor-specific characteristics. Information gleaned from 3D cell culture platforms is considered invaluable, as these platforms provide a controlled environment through which TME conditions can be quantitatively measured for incorporation into mathematical model parameters (100), especially if digital pathology can be leveraged to obtain such measurements. Further, recent advances in omic technologies as well as computational power are expected to pave the way for more detailed integration of GBM cellular metabolism and tissue mechanics, for example, leveraging both machine learning and mechanistic modeling. Nevertheless, much remains to be done to translate results with bioengineering models into clinical practice. Recent mathematical modeling to personalize therapy as well as surgery for GBM based on detailed clinically-obtainable characteristics (e.g. by Swanson et al [101]), has laid the groundwork to move this work toward translation, with the ultimate goal to tailor treatment to patient-specific GBM conditions.

THERAPEUTIC STRATEGIES TO TARGET METABOLIC DYSREGULATION IN THE TME

As the study of GBM metabolism matures, a variety of new therapies including small molecule therapeutics have emerged to provide promising avenues that may bring success where protein molecules and immunotherapies, with their many obstacles to clinical translation, have failed (14). For example, the anti-diabetic drug metformin, already established in terms of safety and tolerance in humans, has been shown to decrease motility and invasiveness of GBM cell lines (102), as well as inhibit the development of resistance to temozolomide (103). Clinical trials examining potential therapeutic utility of metformin in multiple types of cancers are already underway (104). Metformin achieves its effect through multiple mechanisms, including influencing Akt phosphorylation state, fatty acid synthase expression, and GBM metabolic response to hypoxia (102). It may also alter GBM amino acid metabolism, which would enable selective amino acid depletion as potential therapies (11). Diclofenac, a commonly available anti-inflammatory medication that is also thought to impair glycolysis and the cellular efflux of lactate, was shown to have anti-proliferative and anti-migratory effects on GBM cells synergistically when combined with metformin (53). The interest in ketogenic diet and inhibitors of fatty acid oxidation inhibitors as a possible adjunct to other GBM treatments is another example of where understanding of the tumor metabolome has led to potential treatments that can be clinically tested (105). These interventions carry relatively low toxicity and morbidity compared to conventional treatments, i.e. surgical resection, radiation therapy, and chemotherapy with alkylating agents.

New insights into tumor metabolism are driving development of a slew of experimental agents targeting cancers including GBM. Gboxin, an oxidative phosphorylation and complex V inhibitor, eradicates GBM tumor growth by selectively diminishing the heightened proton gradient required by tumor cells, killing primary GBM and inhibiting cellular oxy-

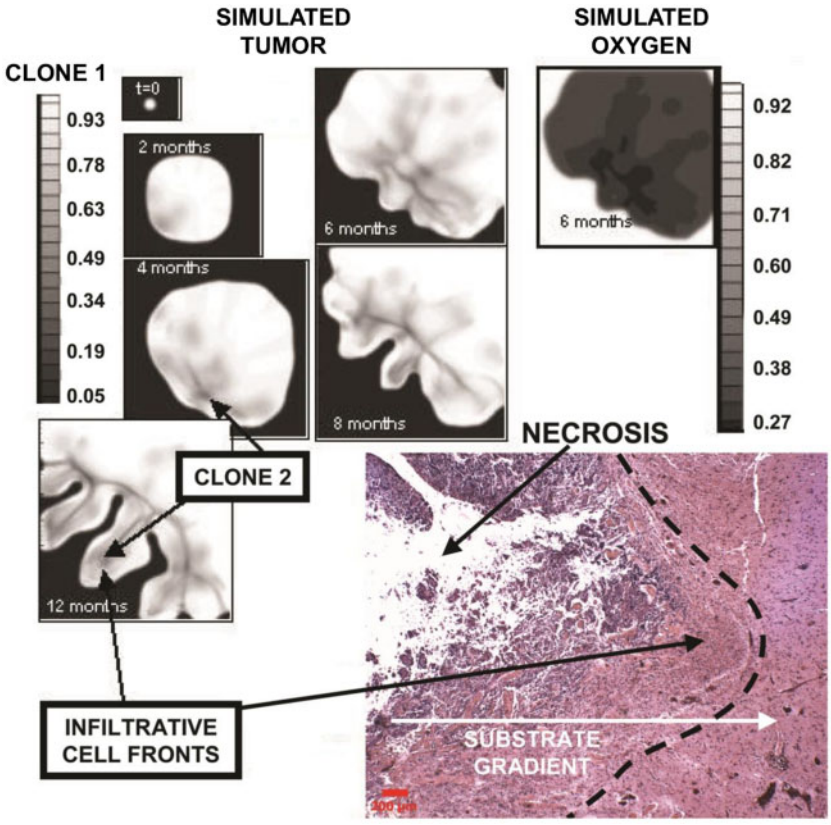

FIGURE 3. A reaction-diffusion mathematical model of tumor growth that hypothesizes functional relationships linking molecular and phenotypic effects, the microenvironment, and tissue-scale growth and morphology identifies and quantifies tumor biologic and molecular properties relating to clinical and morphological phenotype. For different values of the parameters, the model predicts invasion via individual cells, cell chains, strands or detached clusters, as observed in experiments and histopathology, and correlates infiltration morphologies to different stages of progression. The figure shows a simulation of GBM invasion based on differing metabolic activity of tumor cell clones (field of view = 6–10 mm): genotype M = [1,0] (lower-grade clone 1, "C1") evolving to M = [0,1] (higher-grade clone 2, "C2"). Upper left: Local mass fraction of C1. Arrows pointing to darker areas indicate C2. Upper right: Simulated oxygen concentration at 6 months, indicating hypoxic gradients (n = 1 in normal brain and lower in tumor). The larger oxygen uptake of C2 enhances local hypoxia (e.g. bottom left tumor corner), and leads to invasiveness in which clusters of C2 protrude into the tumor mass of C1 first, and into the host brain later. Lower right: Histology section of a tumor front (left) showing an invading cluster into more normal brain (scale bar: 200 μm). A clearly demarcated margin (left of the dashed line) is visible between tumor and more normal brain. Neovascularization and inflammation at the tumor-brain interface is visible as darker spots (to the right) in brain parenchyma, implying that substrate (oxygen, nutrient availability drives collective tumor cell infiltration into the brain). Reprinted with permission from reference (100).

gen consumption in tumor cells and mouse embryonic fibroblasts (106). IACS-010759, a clinical-grade small-molecule complex I inhibitor, decreases proliferation and induces apoptosis in GBM (107). These metabolism-focused therapies show that disruption of metabolic pathways may significantly deter tumor progression and support the view that continued dissection of the GBM metabolic machinery may be key to better targeted treatments.

Concluding Remarks

Detailed study of the interplay between GBM metabolism and the TME may elucidate underlying mechanisms of tumor progression and therapeutic responses, enabling identification of novel therapeutic targets. This understanding has the potential for patient-specific diagnosis and treatment that could improve overall survival. The search for effective therapies requires identification of the most relevant contributors to disease progression with sufficient mechanistic detail that allows discovery of feasibility. Despite tremendous effort, however, the iterative loop of progress, which ideally includes experimental data integrated into computational modeling that then reveals novel insights validated experimentally, remains an ongoing effort. A major reason is that "closing the loop" requires an ongoing interdisciplinary bioengineering perspective that integrates quantifiable experimental data, such as from 3D cell culture systems and digital pathology, with complex mathematical formulations derived from currently limited biological knowledge.

Evaluation of the biomechanical aspects of the TME is leading to discovery of new pieces of the puzzle of GBM biology. The emerging field of GBM mechanobiology is identify-

ing multiple mechanistic points that may be of clinical relevance with evidence of its direct role in modulating GBM proliferation and invasion. Additionally, varying the mechanical stiffness of polyacrylamide gel substrates modulates the fluorogenic metabolite protoporphyrin IX in GBM cell lines, which has implications for the use of 5-ALA fluorescence-guided surgical resection (108). Identification of other emerging molecular targets, such as BMP4, PIEZO1 and CD44 and how they interact with the biomechanical milieu in GBM and control metabolism may aid the development of new therapeutic agents (109, 110). It will be interesting to elucidate biomechanical aspects of TME interactions with the metabolome, as this could advance GBM clinical management.

The TME dynamically supports tumor growth by modulating cancer metabolism to adapt and overcome microenvironmental challenges. Engineering and modeling perspectives based on 3D cell culture and mathematical models offer the possibility of integrated analyses of the TME and GBM metabolism, which could also be extended to other cancer types. Such analyses are expected to pave the way for therapeutic strategies that disrupt metabolic pathways associated with dysregulated GBM microenvironment cues, and thus lead to more clinically successful treatments.

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