Metformin's Therapeutic Potential in Glioblastoma: A Review of its Cellular and Immunomodulatory Effects

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Abstract

Glioblastoma (GBM) is a highly aggressive brain tumor, characterized by its complex tumor microenvironment (TME) and resistance to traditional therapies. Patients with this disease often have a poor prognosis. This review examines metformin's potential as a repurposed therapy, focusing on its dual effects on tumor metabolism and immunity. Preclinical studies demonstrate that metformin inhibits GBM cell proliferation, reduces invasion, and targets glioma stem-like cells. Metformin also modulates the immune TME by shifting tumor-associated macrophages toward a pro-inflammatory M1-like phenotype while suppressing the pro-tumor M2 state. Clinical evidence is emerging but nuanced. A retrospective analysis suggests patients who took metformin and had grade III gliomas survived longer. However, the same effect was not observed in GBMs, suggesting metformin's action could be dependent on tumor genetics. Clinical trials have confirmed the feasibility and safety of using metformin as an adjuvant therapy, though challenges remain in optimizing dosing and patient selection. Metformin offers a promising strategy to reduce tumor growth, invasion, and recurrence. However, future studies are needed to translate preclinical insights into effective clinical interventions.

Keywords: *glioblastoma; metformin; tumor microenvironment; macrophage polarization; glioma stem cells; tumor metabolism; immunotherapy*

Overview of Glioblastoma Tumors

Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumor in adults, accounting for roughly half of all malignant central nervous system tumors (Caverzán et al., 2023). Although GBMs occur almost exclusively in the brain, they can also develop in the brainstem, cerebellum, and spinal cord. GBMs are believed to arise from neural stem cells or glial progenitor cells, depending on the genetic mutations involved (Liu et al., 2011). Current GBM treatments include radiation therapy, surgical resection, and chemotherapy, among others. However, recurrence is high, and despite intensive treatments, most patients have a poor prognosis.

The Tumor Microenvironment in Glioblastomas

Part of what makes GBMs so challenging to treat is the tumor's microenvironment (TME), which plays an active role in driving disease progression, therapy resistance, and immune evasion. For example, the TME evades immune detection by recruiting tumor-associated macrophages and microglia (TAMs). Once inside the tumor, these immune cells are polarized to secrete growth factors, cytokines, and chemokines that ultimately promote tumor invasion, angiogenesis, and immune escape (Hambardzumyan et al., 2016).

Microglia, as well as macrophages, may exist as an M1-like pro-inflammatory phenotype or as an M2-like anti-inflammatory phenotype. The latter produces anti-inflammatory cytokines and growth factors necessary for tissue repair. In this way, M2-polarization promotes GBM growth. In contrast, M1-activation of microglia and macrophages produces a pro-inflammatory response that results in anti-tumor effects. The critical role of microglia and macrophages in GBM pathology provides a strong rationale for exploring therapies that shift these cells' function and polarization status.

Metformin as a Therapeutic Agent

One unexpected candidate in the effort to treat GBMs is the commonly prescribed drug metformin. Metformin is an oral antihyperglycemic agent, the first-line therapy for type 2 diabetes mellitus (Shah et al., 2024). In recent years, metformin's effects have been found to extend beyond glycemic control, with novel antineoplastic and immunomodulatory properties coming to light. For example, some studies have shown that metformin can slow down or inhibit cell growth and interfere with cancer metastasis. These effects have been observed across a wide range of cancer types, including breast, liver, pancreatic, and bone (Lv & Guo, 2020). This review explores how metformin may influence GBM progression and microglial polarization, highlighting its therapeutic potential beyond glycemic control.

Cellular Mechanism

At the cellular level, metformin acts by activating adenosine monophosphate-activated protein kinase (AMPK) and downregulating the mammalian target of rapamycin (mTOR) signaling pathway (Lv & Guo, 2020). It first inhibits mitochondrial respiration, forcing tumor cells into a state of energetic stress. In response, the cells activate AMPK, which in turn suppresses the mTOR pathway. Shutting off the mTOR pathway is a tactic cancer cells use to save energy; however, it can cause cell cycle arrest. In this way, metformin effectively halts tumor cell proliferation and survival (Figure 1).

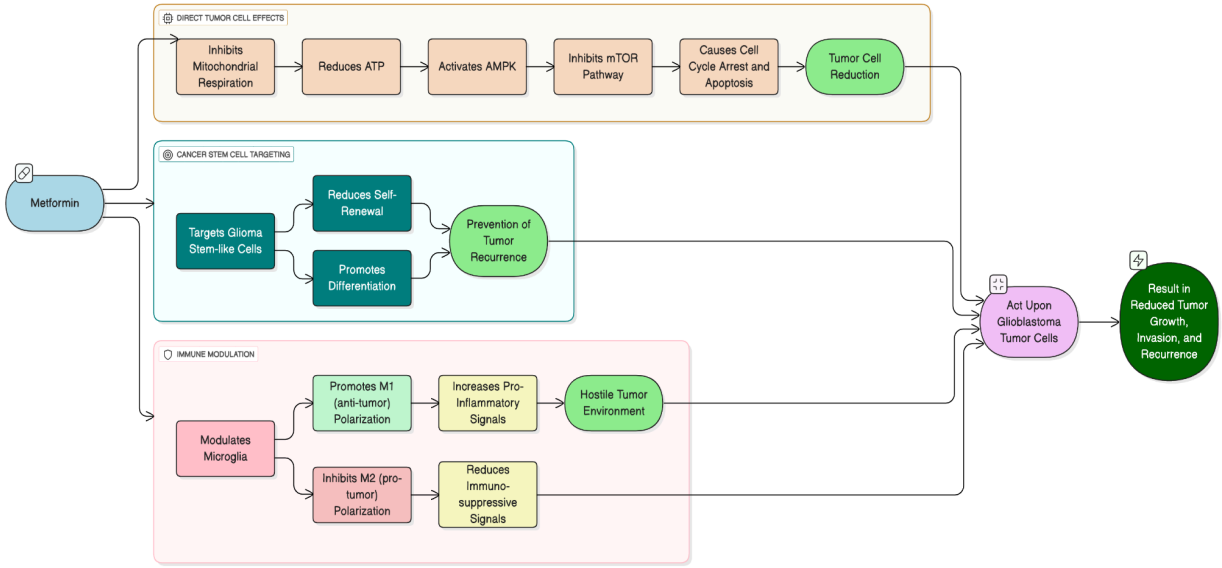

Figure 1. Metformin reduces GBM growth by inhibiting tumor cell viability, targeting stem-like cells, and modulating the immune microenvironment.

Evidence from GBM Cell Line Models

Experiments using GBM cell lines have shown that metformin has antitumor effects. From the discussion above, it can be noted that metformin can suppress tumor cell proliferation and induce apoptosis by interfering with metabolic and signaling pathways. In a recent study, Hong et al. (2024) showed that metformin suppresses GBM cell viability in a dose-dependent manner. The researchers treated cultures of the human GBM cell line U87 with varying concentrations of metformin and observed that higher doses caused more cell death over 72 hours (Hong et al., 2024).

These findings support and extend the work of Sesen et al. (2015), who showed that metformin significantly reduced oxygen consumption and ATP production in GBM cell lines (U87, U251, LN18, and SF767), which led to decreased cell proliferation. Sesen et al. (2015) further demonstrated that metformin induces cell cycle arrest at the G1 phase and increases the

number of cells entering the G0 phase. This mechanism offers a logical explanation for how metformin slows down tumor growth.

Building on these metabolic and antiproliferative effects, other studies have examined metformin's ability to impair GBM cell motility and invasiveness. Al Hassan et al. (2018) reported that treatment with 2.5 mM of metformin reduced wound closure and migration in SF268 GBM cells and decreased migration (but not wound closure) in U87 cells. In the same study, the researchers used a collagen-based invasion assay and found that metformin inhibited cellular invasion by around 30% in SF268 cells and 50% in U87 cells. These observations suggest that metformin may directly impair how GBM cells invade and migrate into surrounding tissues. Taken together, the evidence indicates that metformin can slow tumor development and limit the invasive properties that make GBMs so lethal.

Evidence from GBM Animal Models

With promising results from cell line experiments, researchers are now testing metformin in animal studies to evaluate its efficacy against GBMs. For example, Sesen et al. (2015) extended their in vitro work by injecting mice with the GBM cell lines U87 and LN18. Once tumors developed, the mice received metformin or phosphate-buffered saline injections. Tumors in the metformin-treated group were significantly smaller in weight and volume compared to the controls, highlighting metformin’s potential to slow GBM growth in vivo (Sesen et al., 2015).

Metformin can be used as a standalone therapy to treat GBMs. However, when used in combination with other treatments, its antitumor activity is enhanced. Lee et al. (2018) showed that combining metformin with temozolomide (a standard chemotherapy drug) significantly extended survival in mice bearing GBM xenografts. Moreover, the researchers found that tumors

from treated mice exhibited increased AMPK activation and decreased levels of fatty acid synthase. AMPK regulates cellular energy balance, while fatty acid synthase produces lipids essential for tumor growth. These findings suggest that metformin enhances the efficacy of temozolomide (TMZ) by disrupting tumor metabolism and inhibiting pathways necessary for growth.

More recently, Qiao et al. (2024) found that combining metformin with simvastatin (a cholesterol medication) reduced tumor volume in GBM-bearing mice more than either drug alone. The authors also observed significant reductions in important tumor markers related to energy use and cell proliferation, including GLUT1, GLUT6, and Ki-67 (Qiao et al., 2024). These results imply that metformin and simvastatin together can enhance the inhibition of tumor metabolism and growth. Overall, these in vivo studies demonstrate that combination treatments including metformin offer a more powerful approach to combating GBMs.

Metformin's Effects on Glioma Stem-Like Cells

GBM tumors are highly heterogeneous. Within these tumors exists a subpopulation of glioma stem-like cells (GSCs) that possess the ability to self-renew and differentiate into various tumor cell types. These GSCs are critical in driving tumor initiation, progression, and recurrence. GSCs resist conventional therapies like chemotherapy and radiation, which is part of what makes them so dangerous. Therefore, it is important to target GSCs to improve treatment outcomes and prevent tumor relapse.

Fortunately, a growing body of research has investigated the use of metformin to accomplish this goal. Sato et al. (2012) demonstrated that metformin reduces the self-renewal capacity of GSCs and promotes their differentiation into non-tumorigenic neural and glial cells.

This is likely accomplished through the activation of AMPK and the subsequent FOXO3 pathway. Another interesting finding from this study is that GSCs pretreated with metformin showed a reduced ability to create tumors in vivo, leading to a delay in tumor formation and prolonged survival in mice. These results suggest that metformin can target GSCs by impairing their ability to create and sustain tumors.

Given metformin's ability to target GSCs, researchers then explored its potential when combined with standard chemotherapy agents. Yu et al. (2016) took GSCs isolated from human and rat glioma cell lines (U87 and C6, respectively) and treated them with TMZ and metformin (both individually and in combination). The researchers found that combining TMZ and metformin was more effective at inhibiting GSC growth than using either agent alone. Furthermore, the treatment induced stronger cell cycle arrest (mainly at the G2/M and S phases) and greatly increased apoptosis in GSCs. This study suggests that the combination of metformin and TMZ may improve the effectiveness of therapies targeting these cell types.

Researchers have also explored whether metformin can prevent GBM cells from gaining stem-like features. Song et al. (2018) found that TGF-β1 drives GBM cells to adopt traits that increase their stemness and invasiveness, effectively shifting them toward a glioma stem-like state. Metformin effectively blocked this shift by inhibiting key signaling pathways (protein kinase B [AKT]/mTOR and ZEB1). This action not only reduced the expression of stem cell markers but also limited the formation of tumor spheres. Collectively, these findings suggest that metformin can target existing GSCs and inhibit the formation of new ones by reducing self-renewal and promoting differentiation (Figure 1). These effects offer a promising new avenue to improve GBM treatment and reduce tumor recurrence.

Metformin's Effects on the Polarization of TAMs

The central role of TAMs in GBM progression has led researchers to examine whether metformin can modulate their polarization. The prevailing theory is that metformin shifts these immune cells toward an M1-like phenotype while reducing M2-like markers (Figure 1). TAMs in GBMs frequently exhibit an M2-like, immunosuppressive phenotype. This phenotype contributes to tumor progression, increased invasiveness, and resistance to therapeutic interventions.

Ding et al. (2015) demonstrated that metformin can effectively block this M2-like polarization. The researchers found that metformin-treated macrophages had lower levels of the M2 surface protein marker CD206 and reduced expression of M2-associated genes like *MRC1*, *PPARγ*, and *Arg1* (Ding et al., 2015). The modified macrophages lost their ability to support tumor growth as they no longer supported cancer cell migration or promoted the formation of new blood vessels. This finding was further validated in mouse models, where metformin treatment reduced cancer metastasis only when TAMs were present. This study's findings point to the conclusion that metformin's anti-cancer effects are primarily driven by its ability to block M2 polarization.

The cytokine environment plays a major role in how immune cells (like macrophages) polarize. For instance, IL-4, IL-10, and IL-13 promote M2 polarization, while IFN-γ drives M1 activation (Van Dyken & Locksley, 2013). Building on this notion, Chiang et al. (2017) found that breast cancer cells treated with metformin secreted lower levels of M2-promoting cytokines (IL-4, IL-10, and IL-13) and increased levels of M1-promoting cytokines (IFN-γ, IL-12, and TNF-α) at both the RNA and protein levels. Because of this, macrophages shifted toward the

M1-like phenotype. This shift diminishes signals that promote tumor growth, thereby limiting tumor cell invasion and angiogenesis.

A separate line of research by Geranmayeh et al. (2022) further supports metformin's anti-M2 effects through a distinct mechanism. These researchers found that metformin directly suppressed microglial cells (a subset of TAMs) from becoming the pro-tumor M2 phenotype. They confirmed this by observing a decrease in CD206+ cells in a harsh, nutrient-poor environment that mimicked tumor conditions. This effect was tied to a mechanism involving mTOR signaling, an important pathway that regulates cell growth and is often overactive in tumors.

Early research provides compelling evidence that metformin alters the polarization of TAMs. The diabetic drug changes immune cells by steering them toward a pro-inflammatory, M1-like phenotype. In the context of GBMs, this shift could have profound therapeutic implications, as promoting an M1-like immune response could allow metformin to limit tumor spread and invasiveness.

Clinical Insights and Limitations of Metformin Therapy in GBMs

Several clinical trials have evaluated the practicality and effectiveness of using metformin to treat GBMs in patients. Aside from its antitumor effects, metformin is a favorable drug to repurpose in neuro-oncology because it is relatively safe and low-cost. However, investigations are still in the early stages, and findings should be interpreted with caution.

Seliger et al. (2019) conducted a retrospective analysis showing that diabetic patients with grade III gliomas who were taking metformin had higher survival rates compared to those

not taking the drug. However, this effect was not observed for patients with grade IV gliomas, implying that the survival benefit may be specific to certain tumor subtypes. This distinction is important because the World Health Organization classifies GBMs as grade IV gliomas (Seliger et al., 2019). According to the authors, one explanation for this finding is that grade III gliomas often carry mutations in the isocitrate dehydrogenase (IDH) gene, which alters how tumor cells generate energy. Metformin may further disrupt this energy production and slow tumor growth. In contrast, GBMs are typically IDH-wild types, meaning they lack IDH mutations. Although IDH-wild type GBMs are more common, they are also more aggressive and associated with poorer patient outcomes.

Other studies have tested metformin in combination with standard GBM interventions. Porper et al. (2021) conducted a phase I clinical trial to evaluate the feasibility of combining metformin with radiotherapy in patients with high-grade gliomas, including GBMs. Some patients also followed a modified Atkins diet to support the intervention. The study found that the combination was generally safe and feasible. However, some participants found it challenging to tolerate the highest dose of 850 mg of metformin taken three times daily (Porper et al., 2021).

Building on these results, an ongoing Phase II trial at the University of Milano-Bicocca is evaluating the use of metformin alongside chemoradiotherapy in newly diagnosed IDH-wildtype GBM patients (University of Milano-Bicocca, n.d.). The trial seeks to provide the same intervention to 25 participants. Recruitment is in progress, but this trial is particularly important because it directly addresses a key limitation of prior research by testing whether metformin can provide a benefit to GBM patients when used alongside other therapies.

Another phase I/II clinical trial is being conducted by Narita et al. (2025) to analyze the combination of high-dose metformin (2,250 mg/day) with TMZ in newly diagnosed GBM patients. According to the published abstract, nearly half (47.6%) of patients had no tumor growth one year after starting treatment, and just over half (54.5%) were still alive two years after treatment. This data suggests potential clinical benefits, but the results are still preliminary.

Overall, these studies indicate that metformin shows potential in GBM treatment, though its effects vary by context. Clinical benefit is influenced by factors such as genetics, tumor subtype, and medication schedules. The clinical trials discussed illustrate the difficulties of translating preclinical findings into clinical application. One major issue is dosing, as the high concentrations of metformin shown to be effective in vitro may not be well tolerated by patients. Another limitation lies in patient selection, since metformin is unlikely to provide benefit across all GBM populations.

Conclusion and Future Directions

GBMs are one of the most aggressive and treatment-resistant types of brain tumors. One of the reasons for this resistance lies within the TME, specifically the role played by microglia that promote tumor growth, suppress immune responses, and result in treatment failure. Targeting tumor cells and their surrounding microenvironment is key to improving treatment outcomes.

Metformin shows dual potential in this context. First, it can directly inhibit GBM cell proliferation and reduce stem-like cell populations. Second, it reshapes the tumor immune environment by polarizing microglia into the anti-tumor M1 phenotype and suppressing the pro-tumor M2 state. Shifts in cytokine profiles also accompany these changes. Thus, metformin

can bypass specific resistance mechanisms that tumor cells employ through its action on tumor-intrinsic factors and the surrounding immune microenvironment.

Early clinical trials and retrospective studies indicate that metformin is generally safe and feasible in conjunction with standard therapies. Early findings also show evidence of potential clinical benefits, though results are early-phase and very context-specific. Key hurdles in advancing this therapy include optimizing dosing, selecting patients most likely to benefit, and identifying biomarkers that predict treatment response.

Future research should work to identify tumor subtypes or genetic markers that can predict which patients will derive the greatest benefit from metformin therapy. Researchers should also continue refining dosing strategies and exploring combination treatments to enhance metformin's anti-tumor activity. Although preclinical results are encouraging, the full clinical potential of metformin in GBMs remains to be established. In the long run, these efforts could improve the survival and quality of life for patients facing this devastating cancer.

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