Trends in Molecular Medicine

CellPress

Opinion

Exploiting the gut microbiome for brain tumour treatment

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Increasing evidence suggests that the gut microbiome plays a key role in a host of pathological conditions, including cancer. Indeed, the bidirectional communication that occurs between the gut and the brain, known as the 'gut–brain axis,' has recently been implicated in brain tumour pathology. Here, we focus on current research that supports a gut microbiome–brain tumour link with emphasis on high-grade gliomas, the most aggressive of all brain tumours, and the impact on the glioma tumour microenvironment. We discuss the potential use of gut–brain axis signals to improve responses to current and future therapeutic approaches. We highlight that the success of novel treatment strategies may rely on patient-specific microbiome profiles, and these should be considered for personalised treatment approaches.

Highlights

Increasing evidence suggests that the gut microbiome can influence tumour growth, the tumour microenvironment, and response to therapy.

Emerging evidence suggests a link between the gut microbiome and glioma pathogenesis, with patients with glioma exhibiting changes in the gut microbiome.

The gut microbiome of patients with glioma exhibits reduced short-chain fatty acid–producing bacterial species potentially contributing to the immune cold tumour microenvironment.

Microbially derived metabolites, such as short-chain fatty acids, may regulate pro- and antitumour responses in the glioma tumour microenvironment.

Modulation of the gut microbiome may represent a novel therapeutic strategy for treating patients with glioma.

The cancer microbiome

The human **microbiota** (see Glossary) is a complex ecosystem that symbiotically associates with barrier tissues found in the body [1]. The gut **microbiome** can interact and modulate host responses, especially the immune system [2], and the human gut microbiota consists of approximately 1000 species of bacteria as well as thousands of viruses and a less diverse plethora of archaea and fungi [3–5]. The overall composition of the gut microbiome is dependent on several intrinsic and extrinsic factors, and, as a result, each individual harbours a unique set of microbes [6]. Growing evidence suggests that the composition of the microbiota may underpin susceptibility to a variety of diseases, including cancer, as well as influencing response to treatment.

Indeed, differences in the gut microbiota between individuals may increase susceptibility to certain types of cancer [7], and polymorphic variability in the gut microbiome between individuals can have a drastic impact on cancer phenotypes [8]. It is therefore no surprise that microbiome composition was recently included as an enabling characteristic in the new dimensions of the hallmarks of cancer [9,10]. To date, most of the research indicating a host microbiome–cancer interaction has been conducted in colorectal cancer, breast cancer, or melanoma. In colon cancer, tumour-promoting and cancer-protective microbiomes that can modulate the incidence and pathogenesis of the disease have been described [11]. Similarly, in breast cancer, alterations in the gut microbiome have been observed early in disease development with growing evidence linking these altered signatures with disease progression [12], whereas in melanoma, an intimate connection between the host microbiota and response to **immunotherapies** has recently emerged [13,14].

The gut microbiome and the brain communicate bidirectionally through various pathways collectively termed the '**gut–brain axis**' [15]. Consequently, perturbations in the gut microbiota throughout life have been linked to central nervous system (CNS) disorders and diseases [2,15]. **High-grade gliomas** such as **glioblastoma** are aggressive brain tumours and have one of the poorest prognoses of all cancers, with median survival only in the range of 15 months

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from diagnosis [16]. Emerging evidence suggests a link between the gut microbiome and glioma pathogenesis with patients with glioma exhibiting changes in the gut microbiome. It is not yet clear whether such glioma-related gut alterations precede glioma initiation, and therefore may represent a vulnerability to glioma development, or that the developing glioma induces changes in the gut microbiome via the bidirectional communication that occurs through the gut–brain axis [15] (Figure 1). Nevertheless, it is apparent that glioma-related gut microbiota changes can impact glioma progression, the tumour microenvironment, and response to therapy [17]. If the gut microbiome can have such a drastic impact on glioma, it leads to important questions such as whether we can enhance responses to current and novel therapies in glioma via the manipulation of the gut microbiome and whether analyses of the gut microbiome of patients with glioma should be used to inform treatment selection for a more personalised medicine approach. We will argue that profiling the gut microbiome of patients with glioma will be essential in the future as part of a precision medicine approach.

Evidence linking the gut microbiome to brain tumour pathology

Evidence that there could be a link between **gut dysbiosis** and glioma pathology is still in its infancy, but a growing interest in this field has emerged [18]. Differences in the gut microbiome

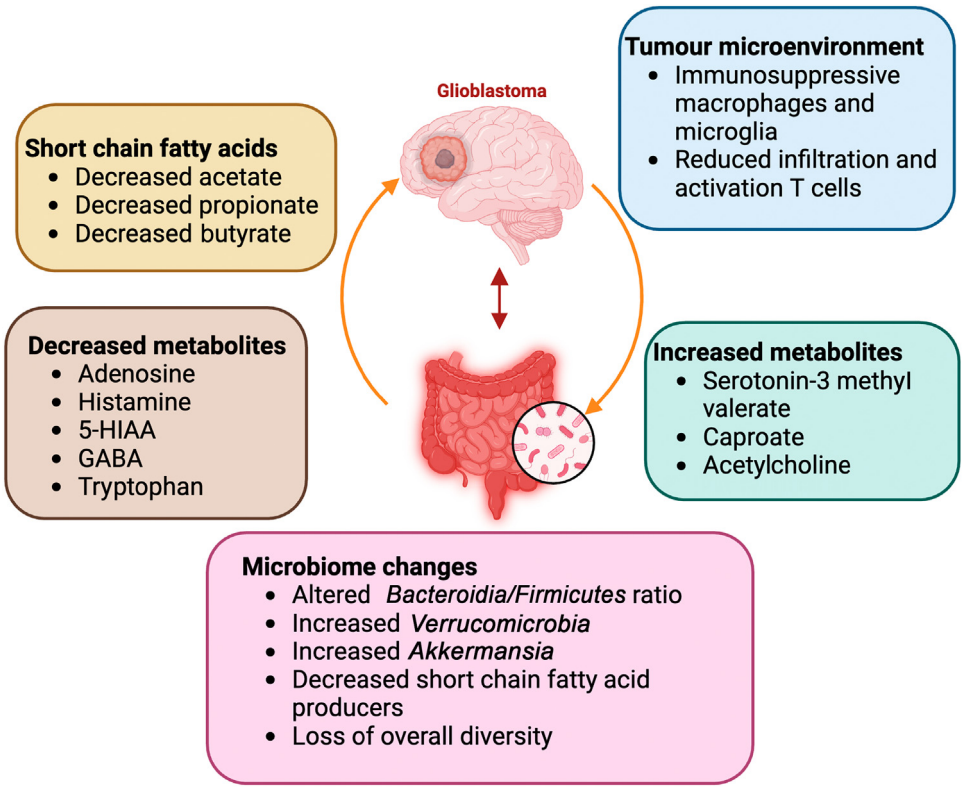

Figure 1. Bidirectional communication between the gut and the brain in glioblastoma. Changes reported to occur in the gut microbiome of patients with glioma are described compared with the healthy human microbiome. The gut microbiome in patients with glioma and humanized mouse models shows a lack of microbial diversity, an increased ratio between *Firmicutes* and *Bacteroides* (F/B), and an increase of both *Verrucomicrobia* on the phylum level and *Akkermansia* on the genus level, compared with healthy controls. As well as alterations in the glioma-associated microbiome, alterations in the levels of short-chain fatty acid (SCFA) and other metabolites have been reported. This figure was created using BioRender (https://biorender.com/). Abbreviations: GABA, γ-aminobutyric acid; 5-HIAA, 5-hydroxyindoleacetic acid.

Glossary

Alpha diversity: a measure of the diversity within an ecological community, considering both species richness and evenness, often indicating ecosystem health.
Beta diversity: a measure of the similarity or dissimilarity between two microbiota communities.
Chemotherapy: a cancer treatment using drugs to destroy rapidly dividing cells, often combined with other therapies.
F/B ratio (Firmicutes/Bacteroidetes ratio): an indicator of gut health, representing the balance between two major bacterial phyla, often linked to metabolic conditions.
Faecal microbiota transplantation (FMT): the transfer of stool from a healthy donor to a recipient's gastrointestinal tract to restore gut bacteria balance.
Genus: a taxonomic rank grouping structurally similar or related species, above species and below family in the biological hierarchy.
Glioblastoma: a fast-growing type of central nervous system tumour that forms from glial (supportive) tissue of the brain and spinal cord and has cells that look very different from normal cells. Also called GBM or glioblastoma multiforme.
Gut–brain axis: the bidirectional communication network between the gut and brain, crucial for regulating various physiological processes.
High-grade gliomas: aggressive brain tumours originating from glial cells, known for their rapid growth and resistance to treatment.
Immune checkpoint blockade (ICB): a form of immunotherapy that blocks proteins used by tumour cells to suppress immune response, boosting the ability of T cells to recognise and attack cancer.
Immunotherapies: treatments that enhance the immune system's ability to recognize and destroy cancer cells.
Ketogenic diet: a high-fat, low-carbohydrate diet that induces ketosis.
Microbial metabolites: compounds produced by microorganisms that influence host health, including inflammation and disease development.
Microbiome: the community of microorganisms, including bacteria, fungi, and viruses, found in a specific environment.
Microbiota: the collection of genomes from all microorganisms presents in a specific environment.

between healthy individuals and patients with glioma has recently been described as well as in tumour-bearing mice implanted with GL261 glioma cells. Compared with healthy controls, mice and patients with glioma exhibited significant differences in **beta diversity** specifically in the ratio between *Firmicutes* and *Bacteroides* (**F/B ratio**) as well as an increase of *Verrucomicrobia* on the **phylum** level and *Akkermansia* on the **genus** level [19]. However, it should be noted that these are relatively crude indices of microbiome function. At the genus level, corroborating results from the previous study, a significant increase in the *Akkermansia* genus from the *Verrucomicrobia* phylum after tumour growth was observed [20] (Figure 1). While these studies suggested an increase in specific microbiota during tumour growth, other studies have reported that patients with brain tumours exhibit an overall loss of diversity in the gut microbiome that may contribute to tumour progression. Indeed, other reports found that the **alpha diversity** indices (Shannon, Simpson, and Chao1) were all significantly reduced in patients with glioma compared with healthy controls [21,22]. However, it should be noted that for some of these studies, the number of patients with glioma compared with healthy controls was small (n = 6) and may not reflect robust consistent differences present in the gut microbiome of patients with glioma. Further studies with greater numbers of patients and controls will be needed to clarify differences. It will also be important in future studies to understand which of these potential changes in the gut microbiome of patients with glioma affects treatment regimens or tumour growth. This information will play a crucial role in personalizing treatment regimens.

The apparent gut alterations in humans and mice have increasingly been linked to glioma development. The finding that *Bacteroidia* are decreased and *Firmicutes* are increased has been associated with accelerated glioma progression [23]. Mice with alterations in the F/B ratio induced by antibiotics and before GL261 tumour implantation had a significant deterioration, further implicating the gut microbiome in glioma growth and progression [23]. However, it should be noted that the antibiotic cocktails such as those used in this study (i.e., ampicillin, vancomycin, neomycin, and metronidazole) will target many species of bacteria and not just those relating to the F/B ratio. The finding that antibiotic treatment can promote glioma tumour growth has been validated in additional mouse models [24,25]. As well as decreased survival in antibiotic-treated mice and enhanced tumour growth, changes in immune cell components were also observed. These changes included reduced cytotoxic natural killer cell subsets [25], altered expression of inflammatory mediators in microglia cells, increased infiltration of Foxp3 regulatory T cells [23], and remodelling of the tumour microenvironment towards a proangiogenic phenotype [24]. These findings collectively demonstrate that changes in the immune component of the tumour microenvironment could contribute to the increased glioma growth observed after antibiotic treatment in mice. Further work will be needed to understand the underlying mechanisms that may regulate immune responses in this context and how they may be regulated through the glioma-associated gut microbiome as well as how these changes could be important when deciding on appropriate treatment regimens for patients.

Microbe-derived signals and modulation of the hot versus cold brain tumour microenvironment

The mechanisms underpinning gut–brain communication are complex and involve the immune system, endocrine system, the autonomic nervous system, and metabolic pathways and have been reviewed extensively elsewhere [15]. Of particular interest are microbe-derived metabolites such as **short-chain fatty acids (SCFAs)**, acetate, propionate, and butyrate [26,27]. SCFAs and other **microbial metabolites** are important regulators of immune system development and activation and have also been implicated more recently in regulating cancer immunity [28]. SCFAs can enter the CNS and can also impact blood–brain barrier (BBB) permeability [29,30],

Phylum: a major taxonomic rank that groups organisms with a shared body plan, positioned above class and below kingdom in the biological hierarchy.
Prebiotic: nondigestible food ingredients that promote the growth or activity of beneficial bacteria in the colon, enhancing host health.
Probiotic: live microorganisms that, when consumed in adequate amounts, confer health benefits to the host.
Radiotherapy: treatment using high-energy ionizing radiation to kill or damage cancer cells.
Short-chain fatty acids (SCFAs): fatty acids produced by gut bacteria during fibre fermentation, important for gut health and immune function.

an important consideration for drug bioavailability at the brain tumour site, potentially impacting efficacy.

Faecal metabolites obtained from glioma-bearing mice exhibit reduced levels of SCFAs, acetate, butyrate, and propionate, as well as decreases in adenosine, histamine, norepinephrine, 5-hydroxyindoleacetic acid (5-HIAA), γ-aminobutyric acid (GABA), and tryptophan. By contrast, increases in the metabolites, serotonin 3-methyl valerate, caproate, and acetylcholine were observed in glioma-bearing mice [20] (Figure 1). In the same study, similar decreases in faecal metabolites 5-HIAA and norepinephrine were observed in patients with glioma compared with controls [20]. Decreases in SCFAs, acetate, butyrate, and propionate were also observed in the caecum of late-stage GL261 glioma-bearing mice in a second study [31]. This consistently observed decrease in SCFA is not surprising, given the loss of diversity of SCFA-producing bacterial species in glioma as previously mentioned [21] (Figure 1). Could the loss of SCFA metabolites or SCFA-producing bacteria affect the growth of the glioma or how it responds to certain treatments? Could profiling SCFA represent a novel biomarker to inform treatment strategies for patients with glioma? Future work will be needed to understand if profiling these metabolites/microbes could be beneficial for patients with glioma to inform better treatment strategies.

It is also unknown how the loss of SCFA metabolites could affect the tumour microenvironment in the context of glioma-associated macrophages/microglia (GAMs) (Box 1 and Figure 2) or in the context of T cell immunity (Figure 2). While gliomas have considerable infiltration of GAMs, they are considered 'immune cold' tumours, meaning they have low T cell infiltration as well as having one of the lowest rates of tumour mutational burden (TMB), resulting in fewer cancer-specific neoantigens and overall poor immunogenicity and response to immunotherapies [32,33]. SCFAs have been shown to be essential in shaping both effector and regulatory T cells through epigenetic and metabolic reprogramming [34]. Indeed, butyrate is important for the expansion and effector function of cytotoxic T lymphocytes (CTLs) in antiviral immunity [35] or in promoting antitumour immunity [36]. Other reports, however, have suggested that the presence of butyrate or butyrate-producing bacteria could limit antitumour responses following irradiation [37]. Therefore, careful consideration will need to be given to when and if SCFA supplementation could be beneficial in the context of glioma treatments, and further work to better understand how the

Box 1. Glioma-associated microglia and macrophages (GAMs) and the gut microbiome

It has been shown, in high-grade glioma, that these non-neoplastic cells are predominantly glioma-associated resident microglia (GAM microglia) or infiltrating bone marrow–derived macrophages (GAM macrophages), collectively known as GAMs [71]. GAMs promote tumorigenesis in glioblastoma by secreting factors important for tumour cell survival and proliferation [72–74]. As a result, efforts to target GAMs in this context using depletion and reprogramming are ongoing [72]. However, these strategies and consequent clinical trials have been disappointing, suggesting we lack knowledge regarding the complexity of GAM recruitment and activation. Of significant interest is the potential to use the gut–brain axis to potentially polarise GAMs to antitumour activation states (see Figure 2 in the main text). It has been shown that microbially derived signals such as metabolites in the form of short-chain fatty acids (SCFAs) regulate macrophage function in health [75,76] and in the context of cancer [77]. Increasing microbiota diversity by high-fibre diet shifted myeloid cells to a more immunostimulatory activation state, whereby absence of the microbiota led to protumoural macrophage activation states [77]. Similarly, it has been established that the gut microbiome has a major impact on microglial processes [78,79]. Germ-free mice exhibit global defects in microglia, including altered cell numbers, defects in maturation, altered metabolic features, changes in morphology and activation, as well as an impaired ability to respond to infection [78]. The major influence of the microbiome on microglial development and function throughout the lifespan has been further substantiated by additional studies [80,81]. Similar to macrophages, these processes appear to be regulated by microbially derived SCFAs, specifically acetate. Bacteria-derived acetate modulates key metabolic processes of microglia at steady state and could rescue impaired microglial maturation in germ-free mice [79]. Microbe-derived SCFA modulation of microglial activation has also enhanced disease pathology in Parkinson's disease [82] and Alzheimer's disease [79,83]. Collectively, these studies highlight the potential of microbially derived metabolites to reprogram microglia and macrophages to specific activation states, and further investigation in the glioma context is warranted.

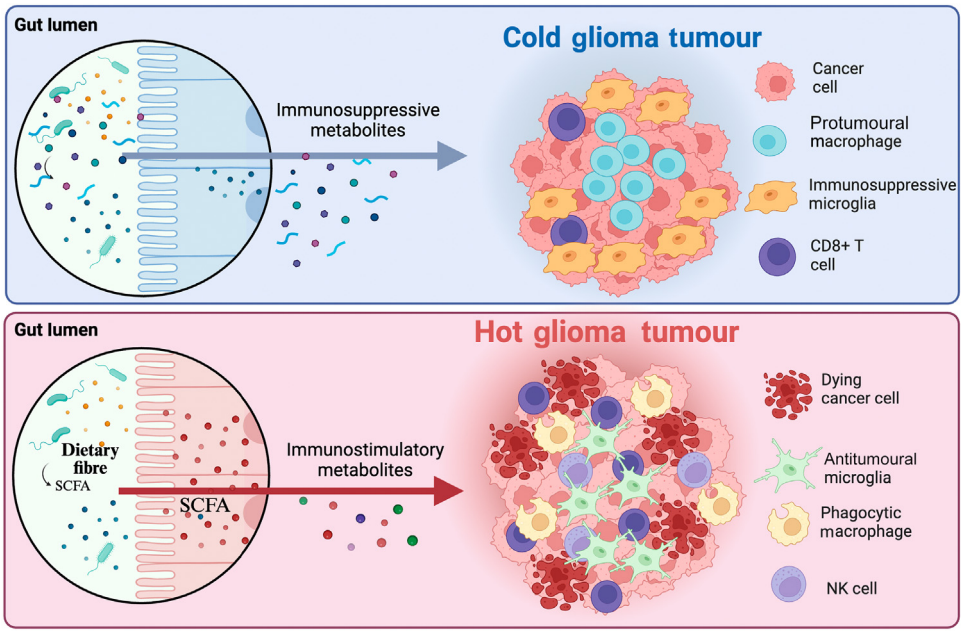

Figure 2. Harnessing the influence of the gut–brain axis to impact glioma cells as a therapeutic strategy. Here we illustrate a hypothetical mechanism postulating how the glioma-associated gut microbiome could influence the immune cold microenvironment in glioblastoma with the potential to 'heat up' the tumour microenvironment. Faecal microbial transplant (FMT), dietary changes, or probiotics could alter the gut microbiome sufficiently to cause intrinsic glioma cell changes via the gut–brain axis. These interventions could improve gut microbial diversity, leading to increased short-chain fatty acid (SCFA) production or altering immunosuppressive factors that hinder therapies such as checkpoint inhibitors. This strategy could bypass the issue with blood–brain barrier penetrance impacting adequate drug delivery and take advantage of the body's innate messaging system or glioma-associated microglia (GAMs). This figure was created using BioRender (https://biorender.com/). Abbreviation: NK, natural killer.

gut microbiome responds to standard treatment regimens such as **chemotherapy** and radiation will be vital (see Clinician's corner).

Gut microbiome and response to current glioma treatments

Response to current glioma treatments is limited. Standard of care for high-grade glioma after maximal safe surgical resection consists of concurrent temozolomide (TMZ) chemotherapy and **radiotherapy** (60 Gy/30 fractions) for 6 weeks, followed by adjuvant TMZ for 6 months [38]. However, despite this aggressive, multifaceted treatment strategy, the tumour inevitably returns and progresses quickly. To date, there is limited literature on the impact of the gut microbiome on cranial radiotherapy in the context of glioma; however, a bidirectional interaction existing between radiotherapy and the gut microbiome has been shown for other cancer types [39]. Exposure to ionising radiation may disrupt the microbiome, and these disruptions can impact the effectiveness of other anticancer treatments [40]. However, we are far from understanding these interactions in the context of brain tumour treatment, and we should be profiling how the gut microbiome changes in patients with glioma following standard therapies such as chemotherapy and radiation.

There is a growing appreciation that chemotherapeutic agents can have direct effects on microbiome composition and that the microbiome can alter the efficacy and toxicity of certain cancer therapeutics [41]. TMZ is an orally administered second-generation alkylating agent that requires physiologic conversion to its active component monomethyl triazenoimidazole carboxamide (MTIC) in the gut. The abundance of certain gut bacteria species, particularly induction of *Akkermansia* and *Bifidobacterium*,

have been linked to the antitumour effect of TMZ and related microbial metabolites significantly correlated with TMZ pharmacodynamic indices [42]. Further verification suggested that gut microbiota depletion by antibiotics could accelerate glioma development, attenuate TMZ efficacy, and inhibit immune cell recruitment of macrophage and CD8α$^+$ T cells [43]. Commonly used second-line therapies such as bevacizumab or lomustine and approaches such as **immune checkpoint blockade (ICB)** have not shown an overall survival benefit in glioma treatment [44–46].

Changes in the microbiome can impact blood–brain barrier permeability and drug bioavailability through the gut–brain axis [47] and could also impact tumour vascularity and tumour vascular permeability. This has been seen with other intrinsic proangiogenic molecules such as von Willebrand factor (vWF) [48], and *Clostridioides difficile* toxins [49]. By similar mechanisms, altering the microbiome could potentially impact the response of patients being treated with angiogenic inhibitors, such as the VEGF inhibitor bevacizumab.

While responses to novel therapeutic strategies such as ICB have been disappointing in the context of glioma, it is unknown if failure of these treatments could relate to changes in the gut microbiome and potential loss of immunostimulatory capacity. Therefore, harnessing the power of the gut microbiome to improve response to current glioma therapies or more novel approaches such as immunotherapy is significant to patients and is discussed in more detail later.

Microbiota-targeted therapeutic opportunities to enhance treatment success in high-grade glioma

Emerging evidence suggests that modulation of the gut microbiota could influence the efficacy of certain cancer therapies. In this section, we briefly discuss three potential microbiota-targeted therapeutic strategies, including dietary interventions, **faecal microbiota transplant (FMT)**, and **probiotics** (Figure 3). Where possible, we provide direct evidence of these strategies being used to restrict glioma growth or enhance responses to glioma-associated treatments. In other instances, we present findings from research in other cancer types where the data might be extrapolated to glioma and argue that profiling the gut microbiome of patients with glioma will be essential for better precision medicine approaches in the future.

Diet

Diet is one of the most important factors influencing microbiota composition. Studies examining a potential association between diet and glioma risk have shown contrasting results, and few to date have focused on specific microbiota-targeted approaches [50,51]. A systematic review quantitatively evaluating the association between various dietary intakes and the risk of developing glioma found that tea, orange vegetables, and green vegetables were all associated with a lower risk of glioma, whereas the intake of grains, processed meat, or fish was associated with increased risk of glioma [50]. However, there is a lack of uniformity of these findings as combined analysis of three large prospective studies found little, if any, association between major food groups or dietary patterns and glioma incidence [51]. This could be due to the caveats associated with systemic versus prospective studies. Although systematic reviews offer a broad overview of several studies, they may include weaker, less controlled studies that might influence conclusions, while prospective studies are generally more controlled but are also limited to the specific population being studied. Further studies have examined if certain diets can modulate glioma progression, with most clinical and preclinical glioma studies focusing on the **ketogenic diet**, to limit glucose availability for glioblastoma tumour cells. In preclinical studies, the implementation of the ketogenic diet reduced tumour size but with limited effects on overall survival [52]. To date, while proven safe and tolerable, it has been difficult to assess clinical efficacy of the ketogenic diet due to small sample sizes [53,54]. Dietary restriction of the amino acids cysteine and methionine

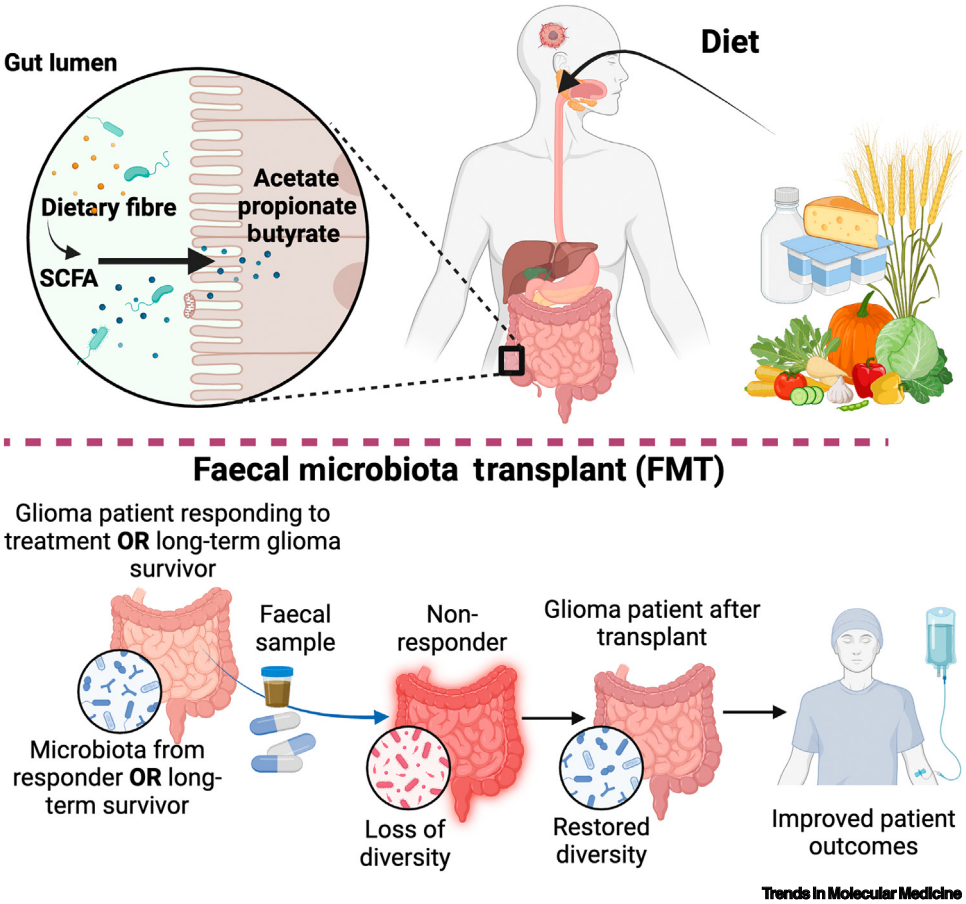

Figure 3. Therapeutic strategies to potentially target the gut microbiota in glioblastoma. Faecal microbiota transplantation (FMT) from long-term glioma survivors or patients responding to novel therapeutics, probiotic or prebiotic strains, or dietary interventions could all replenish the gut microbiome to healthy levels by reducing the lack of diversity or rebalancing microbial strains. This could then stimulate antitumour immunity or restrict tumour growth, improving the efficacy of current or novel treatment approaches used to treat patients with glioma. This figure was created using BioRender (https://biorender.com/). Abbreviation: SCFA, short-chain fatty acid.

were shown to synergise with glutathione peroxidase 4 (GPX-4) inhibitors to prolong survival in orthotopic animal models [55], whereas mice fed an obesity-inducing high-fat diet (HFD) resulted in a hyperaggressive disease and shortened survival compared with a low-fat diet [56]. Collectively, these studies suggest that modulation of diet could impact glioma pathology; however, so far, the use of diet to modulate the gut microbiome to enhance current treatment responses in patients with glioma is relatively unexplored (Figure 3). This should be prioritised in future studies, as reports in other cancer types indicate that high-fibre diets could be beneficial in increasing the responses to ICB [57] or in sensitising tumour cells to radiation [58]. Interestingly, there is limited data on dietary **prebiotics** to date in brain tumours despite evidence suggesting their anticancer roles in other types of cancers [59]. Taken together, much more research is needed in this area to really understand if dietary interventions could be beneficial for patients with glioma.

FMT

The use of ICB for cancer treatment has gained significant interest over the past two decades. The best characterised to date is the PD-L1/PD-1 axis expressed on tumour cells and T cells, respectively [60]. The binding of PD-L1/PD-1 prevents T cells from killing tumour cells, effectively

suppressing immune responses. However, despite substantial effort to develop ICB regimens for glioblastoma treatment, no survival benefit so far has been seen above standard chemotherapy alone [61]. In melanoma, treatment with anti-PD-1 monoclonal antibodies led to long-term clinical benefit in 40% of patients [60]. A significant association was observed between microbial composition and clinical response to anti-PD-1 [62], with responders having significant differences in diversity and composition from non-responders [63]. Importantly, microbiota from initial responders to anti-PD1 therapy was transplanted to non-responder patients, known as FMT [13,14] (Figure 3), leading to favourable changes in the immune landscape as well as clinical responses in previous non-responders [13,14]. In pancreatic cancer, a relationship between the microbiome and long-term survival has been observed, with long-term survivors exhibiting a much more diverse microbiome [64]. Similar to melanoma studies, FMT from long-term survivor gut microbiomes affected tumour growth and survival in animal models of pancreatic cancer [64]. While to date these types of FMT studies have not been carried out in the context of human or mouse glioma, one study transferred faecal matter from five healthy human donors into glioma-bearing mice and investigated their individual growth responses [65]. Interestingly, although similar patterns of GL261 glioma growth were observed between the five donors, there were significant differences in responses to anti-PD-1 [65] corresponding to responders and non-responders. These responses correlated with increased cytotoxic and interferon(IFN)-γ T cell activation as well as specific colonisation of the microbiome *Bacteroides cellulosilyticus* [65]. This important study highlights that the individual microbiome could influence the response of patients with glioma to immunotherapy and would support the notion that microbiomes should be sampled in patients with glioma to inform future treatment strategies. However, this study did not examine if FMT from the responders could convert non-responders in this context, similar to what has been shown in melanoma, nor did it examine if reconstituting *B. cellulosilyticus* into non-responders could boost responses to anti-PD1 (Figure 3). Similar to pancreatic cancer, approximately 1% of patients with glioblastoma exhibit long-term survival of at least 10 years [66]. It would also be important to understand if increases in gut microbiota diversity could be partially responsible for long-term survival and if FMT transfer from long-term survivors could offer any therapeutic benefit (Figure 3). It would be of particular interest to compare the microbiome of long-term survivors when they are first diagnosed with short-term survivors in order to identify potentially beneficial bacterial species that later inform beneficial probiotics as discussed later.

Probiotics

Evidence that modulation of the gut microbiome affects glioma growth and progression could lead to use of probiotics to inhibit tumour growth (Figure 3). Probiotics are live bacteria that are associated with maintaining balance in the gut microbiota. *Lactobacillus plantarum* and *Bifidobacterium lactis* are two common probiotic strains, previously associated with numerous health benefits, and when combined were shown to significantly reduce tumour volume and increase survival in the GL261 glioma mouse model through suppression of the phosphoinositide 3-kinase(PI3K)/AKT growth pathway [67]. In a follow-up study by the same group, a combination of different *Bifidobacterium* strains, notably *Bifidobacterium breve*, *Bifidobacterium longum*, *B. lactis*, and *Bifidobacterium bifidum*, led to similar decreases in tumour size and beneficial survival as well as increasing α-diversity in the gut microbiome [68]. While these studies are encouraging, it is not yet clear if similar effects would be translatable to human glioma. Although currently unexplored in the context of glioma, the use of probiotics such as *Bifidobacterium* has also been associated with enhanced antitumour immunity in melanoma and improved responses to ICB. Given the role of the tumour microenvironment in glioma progression and the apparent effects of antibiotic treatment on the immune cell populations in glioma tumours, it is interesting to speculate that probiotic bacteria such as *Bifidobacterium* could boost responses to immunotherapies, which have so far been disappointing in clinical trials [69,70].

Clinician's corner

The treatment of high-grade glioma remains a major challenge due to the difficulty in delivering therapeutics across the blood–brain barrier and a lack of adequate effective therapeutic strategies. As a result, patient outcomes and prognosis for high-grade patients with glioma have not improved in line with advances recently seen in other cancer types, and the prognoses of these patients remains poor.

Alterations in the gut microbiome have been increasingly linked with glioma development and progression. Harnessing the influence of the gut–brain axis to impact glioma cells is an intriguing therapeutic strategy that warrants further investigation. This strategy could bypass the issue with blood–brain barrier penetrance impacting adequate drug delivery and take advantage of the body's innate messaging system or glioma-associated microglia.

The clinical success of transplanting faecal matter from patients with melanoma responding to immunotherapy to non-responders highlights the incredible potential of the gut microbiome to affect these types of therapies. Faecal microbial transplant (FMT), dietary changes, or probiotics could potentially have similar affects in glioma where poor responses to immunotherapies have been observed, but only in a minority of patients. These interventions could improve gut microbial diversity, leading to increased short-chain fatty acid (SCFA) production or altering immunosuppressive factors that hinder therapies such as checkpoint inhibitors.

Alongside current therapies for glioma, a novel therapeutic strategy focused on altering the gut microbiome could impact glioma cell tumour vascularity and/or vascular permeability or by increasing the efficacy of other therapeutic strategies such as immunotherapy or radiation therapy.

The success of such novel combination treatment strategies may rely on patient-specific microbiome profiles, and these should be considered for personalised treatment approaches in the future.

Concluding remarks

Initial preliminary studies suggest a role for the gut microbiome in glioma pathology and response to treatments such as chemotherapy and radiation. Nonetheless, there is still much to be learned, particularly in understanding the mechanisms by which the gut microbiome influences the glioma tumour microenvironment through microbially derived signals such as metabolites (see Outstanding questions). More studies are needed whereby the microbiome is sampled prior to and over the course of the disease and current treatment regimens to really untangle the various endogenous and extrinsic factors that could regulate antitumour responses. A deeper understanding of gut–immune–tumour interactions could inevitably pave the way for deriving gut microbiome–targeted interventions that could enhance efficacy to current and future glioma therapies. However, overall, we argue that precision medicine approaches would greatly benefit from profiling the gut microbiome of patients with glioma before treatment regimens start. Indeed, the success of novel treatment strategies may rely on patient-specific microbiome profiles, and, therefore, these should start to be considered for personalised treatment approaches. Finally, although the use of preclinical glioma animal models has been invaluable to glioma research, particularly in testing novel therapeutics, much more work is needed in human patients with glioma and respective controls to advance this field in coming years. Profiling the gut microbiome and associated metabolites in conjunction with immune phenotyping in human glioma patient faecal, blood, and tumour samples could greatly enhance our understanding in this context as well as eventually allow the tailoring of therapeutic strategies based on associated profiles.

Outstanding questions

Are there bacterial species in an individual's microbiome that confer susceptibility to developing high-grade glioma, or does a developing glioma induce changes in an individual's gut microbiome?

How does the gut microbiome regulate the glioma tumour microenvironment, and can these interactions be therapeutically manipulated to induce antitumour responses through probiotics, diet, or FMT?

Could the gut microbiome, if sampled at baseline before therapy, predict treatment responses in patients with glioma to current or novel therapies? Should we be sampling each patient microbiome as standard practice to inform treatment?

Could the gut microbiome also influence the development, progression, or response to treatment in other high-grade gliomas such as paediatric diffuse midline gliomas (DMG)?

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Declaration of interests

The authors declare no competing interests.

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