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Gut microbiota and their influence in brain cancer milieu

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Abstract

Microbial communities are not simply remnants of the past but dynamic entities that continuously evolve under the selective pressures of nature, reflecting the intricate and adaptive processes of evolution. The microbiota residing in the various regions of the human body has numerous roles in different physiological processes such as nutrition, metabolism, immune regulation, etc. In the zeal of achieving empirical insights into the ambit of the gut microbiome, the research over the years led to the revelation of reciprocal interaction between the gut microbiome and the cognitive functioning of the human body. Dysbiosis in the gut microbial composition disturbs the homeostatic cognitive functioning of the human body. This dysbiosis has been associated with various chronic diseases, including brain cancer, such as glioma, glioblastoma, etc. This review explores the mechanistic role of dysbiosis-mediated progression of brain cancers and their subtypes. Moreover, it demonstrates the regulatory role of microbial metabolites produced by the gut microbiota, such as short-chain fatty acids, amino acids, lipids, etc., in the tumour progression. Further, we also provide valuable insights into the microbiota mediating the efficiency of therapeutic regimens, thereby leveraging gut microbiota as potential biomarkers and targets for improved treatment outcomes.

Keywords Gut microbiome, Gut brain axis, Brain cancer, Glioma, Glioblastoma, Tumour micro-environment, Metabolites, Immune modulation, Probiotics

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Background

From the ancient aphoristic sagacity of Hippocrates, stating "all diseases begin in the gut", to the modern medical research emphasising the importance of the gut in various aspects of human health, the intestinal microbiome has always played an essential role in the understanding of the healthy functioning of the human body [1, 2]. The beneficial microorganisms, known as probiotics, colonise the gut, where they exhibit various crucial functions such as fermentation of dietary fibres and other undigestible food components that further lead to the production of short-chain fatty acids (SCFAs), enhancing barrier functions of the intestine and immune response regulation by generating anti-inflammatory and immunomodulatory components [3–6]. Along with these functions, they also help in the competitive exclusion of pathogenic microorganisms and the production of antimicrobial substances, which benefit other parts of the host as well [7]. For these microbial communities to exhibit dynamic beneficial activity, sustainment and colonisation in the gut is highly essential [313] [8]. The process commences in the prenatal stage with the translocation of maternal microbiota via the amniotic fluid and placenta within the uterine environment. This is subsequently influenced by the mode of delivery at birth, followed by postnatal nutritional factors, wherein breast milk, enriched with human milk oligosaccharides, plays a crucial role in fostering the establishment of the infant's intestinal microbiota. This developmental trajectory is further shaped by the long-term dietary regimen, encompassing dietary fibres, resistant starch, and other saccharides, which contribute to the continuous modulation of the gut microbiome (Fig. 1) [9–14, 313].

Studies from the last few years have identified the role of gut microbiota in human brain health and disease [15–19]. The core of this relationship between the gut and the brain is the gut-brain axis (GBA), which moderates the bidirectional communication between these two organs in the human body [20]. The principal components of GBA intercommunication entail the central nervous system (CNS), the peripheral nervous system (PNS), including the autonomic nervous system (ANS) and the enteric nervous system (ENS), the vagus nerve, the neuroendocrine hypothalamic pituitary adrenal (HPA) axis, together play integral roles in this bidirectional signalling network. These interactions are mediated through various molecular pathways and metabolites, including the SCFAs, butyrate, propionic acid, valeric acid, peptidoglycans, tryptophan, branched-chain amino acids, etc [21–23]. It is evident that the gut microbiome plays a key role in maintaining the homeostatic functioning of the brain, and any imbalance in the gut microbiome would affect brain health. This state of homeostatic imbalance of the gut microbes is called dysbiosis [24]. It occurs due to several reasons, including intrinsic factors like genomic background, health condition of the individual and extrinsic factors such as xenobiotics in the body, diet, and environmental factors [24, 25]. Dysbiosis is also associated with many ailments, such as irritable bowel syndrome, obesity, insulin-dependent diabetes mellitus, autism spectrum disorders and cancers of the colon, stomach, etc [26–30]. In the process of understanding and utilising various probiotics and their metabolites

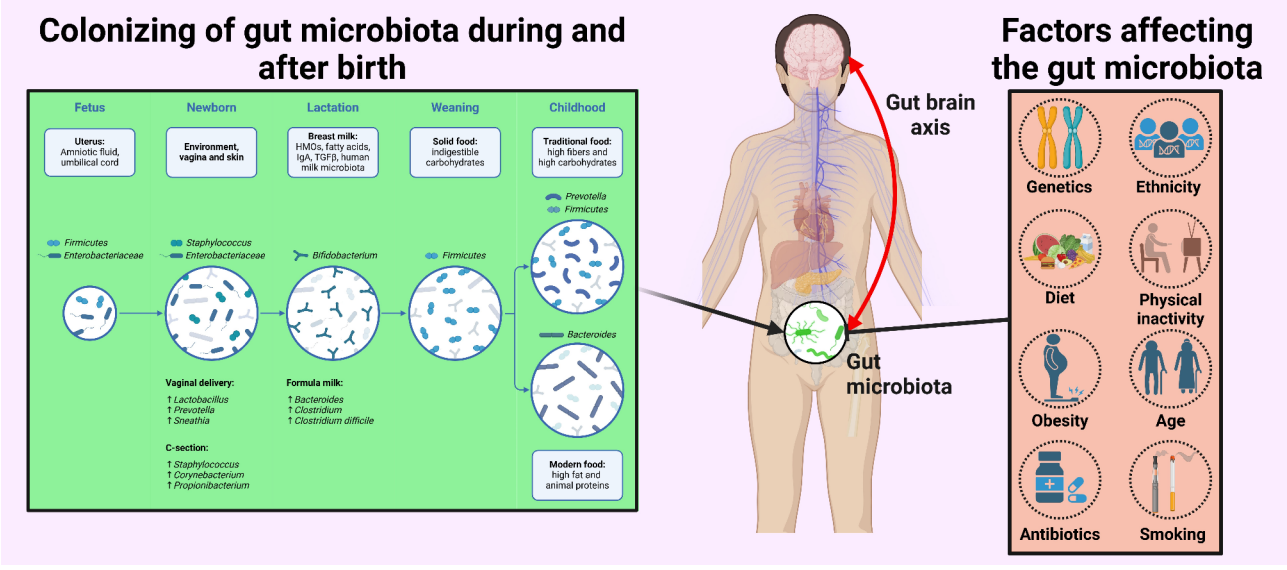

Fig. 1 The stages of colonising the gut microbiome and various factors affecting it: The gut microbiome is initially colonised during the different stages and modes of birth and stages of child life. The factors such as genetics, ethnicity, diet, physical inactivity, obesity, age, smoking and antibiotics affect the health and composition of gut microbiota [313]. This figure was created with BioRender.com

in benefiting human health, several terms representing numerous concepts related to the field of probiotics have emerged, such as prebiotics, synbiotics, postbiotics, psychobiotics and paraprobiotics [31–34]. An emerging concept in the context of GBA is psychobiotics, which were initially defined as "live organisms which, when consumed in adequate amounts, provide a health benefit in patients suffering from psychiatric illness" [31]. It was then expanded to envelop the prebiotics as well [35, 36]. Hence, it is understood that the gut microbiome, as a whole, supports and communicates with the brain for the homeostatic functioning of the human body.

Brain cancer, one of the rarest cancers, ranks 19th in terms of incidence, with 321,476 new cases and 12th in terms of mortality, with 248,305 deaths worldwide, according to GLOBOCAN 2022 [37]. These cancers are unique and have a complex histology with over 100 types as classified by the World Health Organization (WHO) based on histological and molecular parameters [38]. Gliomas account for almost 30% of primary brain tumours and 80% of all malignant ones [39]. The WHO has classified glioma based on its histopathological features as low-grade gliomas (grade I & II), anaplastic (grade III), and glioblastoma (GBM) (grade IV), indicating different malignant stages [40]. GBM, a most frequent and aggressive subtype of glioma, comprises up to 50% of cases, originating from glial cells and can arise spontaneously as primary GBM or develop from a lower-grade or anaplastic astrocytoma, termed secondary GBM [41–45]. Primary GBMs usually exhibit epidermal growth factor receptor (EGFR) upregulation, phosphatase and tensin homolog (PTEN) mutations, p16 deletions and rarely MDM2 proto-oncogene (MDM2) amplifications that develop in older patients [41, 46, 47]. However, secondary GBMs often exhibit tumor protein p53 (TP53) mutations that develop in younger patients [41, 48]. Integrated analysis of genetic alterations in signalling pathways across 33 cancer types from The Cancer Genome Atlas (TCGA) revealed that GBM had a frequency of 86% in cell cycle, 77% in receptor-tyrosine kinase (RTK)/rat sarcoma (RAS), 57% in phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), and 48% in p53 pathways [49]. Low-grade glioma with iso-citrate dehydrogenase (NADP (+))– wildtype (IDH-WT) had a frequency of 82% in RTK/RAS, 64% in the cell cycle, and 47% in PI3K pathways. Low-grade glioma with IDH-Mut-codel had a frequency of 99% in Hippo, 66% in wingless-type MMTV integration site family (Wnt), 50% in transforming growth factor beta (TGFβ), and 45% in cell cycle pathways. Low-grade glioma with IDH-Mut had a frequency of 92% in p53 and 92% in Hippo pathways [49]. The risk factors for brain cancer are genetic factors such as gene mutations, genetic ancestry, and telomere length, demographic factors, namely age, sex, race/ethnicity, and socioeconomic status, and non-genetic risk factors, including allergies, anthropometric factors, head injury, diet, ionizing radiation, organic solvents, smoking, viral infection, structural birth defects, and birth weight etc [50–57]. The therapeutic modalities used to treat GBMs are surgery, chemotherapy, especially temozolomide, radiotherapy, targeted therapy (bevacizumab), immunotherapy, vaccine therapy, virotherapy, and focused ultrasound therapy [58, 59].

Despite the availability of these therapeutic approaches, the clinical outcomes for patients have not improved, and adverse side effects remain a concern [60]. Therefore, alternate strategies are needed to enhance the therapeutic regimen for GBM. Addressing the challenges and issues related to the failure of conventional treatment modalities would lead to the improvement of clinical management of this disease. Cancer Research UK (CRUK) has listed the key challenges that need to be addressed to improve therapeutic efficiency, eventually leading to the cure of brain cancer. One aspect involves comprehending the characteristics of the intricate tumour microenvironment (TME) to ascertain how its constituents can influence the effectiveness of different therapeutic approaches [61]. Brain TME consists of numerous components, including non-cancerous cells such as neurons, oligodendrocytes, astrocytes, pericytes, endothelial cells, and fibroblasts, resident immune cells such as tumour-associated macrophages (TAMs), tumour infiltrating lymphocytes, and microglia and non-cellular components namely cellular signalling molecules, exosomes, secreted extracellular matrix (ECM) remodelling enzymes and other ECM components [62, 63]. Further, it has been reported that GBM demonstrates three unique functional and morphologic esoteric niche such as perivascular, hypoxic, and invasive [64]. Notably, the blood-brain barrier (BBB) also has a significant role in the successful treatment of brain tumours [65]. The heterogeneity in the TME enables it to exhibit various tumour-promoting functions such as immune evasion, cell survival, physical barriers, etc., ultimately leading to the development of multidrug resistance in tumour cells [66]. This drug resistance demonstrates the evolutionary capacity of tumour cells and the crosstalk between tumour cells and the microenvironment under selective therapeutic pressure [67]. The gut microbiota has been shown to regulate the various components of the TME, such as dendritic cells, tumour-associated neutrophils, TAMs, myeloid-derived suppressor cells, cancer-associated fibroblasts, cytokines, metabolites, metabolic and immune reprogramming, genotoxins, and signalling pathways that further regulates tumour progression [68]. It has also been reported that the polymorphic microbiomes, characterised by the diversity and variability of the abundant microorganisms, expedite the acquisition of other additional hallmarks by cancer cells [69]. To shed light on the understanding of

the TME in brain cancer, this review explores the mechanistic regulation of the GBA in brain cancer subtypes, which would also contribute to Sustainable Development Goal 3, that stands for Good Health and Well-Being. The literature search was conducted using the PubMed database to ensure a comprehensive and scientifically rigorous review. The search strategy incorporated specific keywords, including "*gut microbiota*, *gut microbiome*, *gut-brain axis*, *brain cancer*, *glioma*, *glioblastoma*, *microbial metabolites*, *and tumour microenvironment*". Studies were selected based on their relevance to the interplay between gut microbiota and brain tumours, with an emphasis on various brain cancer subtypes. Only peer-reviewed articles published in English were considered, including preclinical studies, clinical trials, and relevant meta-analyses. Studies not directly related to brain tumours, non-peer-reviewed publications, and articles in language other than English were excluded. Studies published in English were included to ensure accuracy in data interpretation and synthesis, as well as to maintain consistency in the analysis. Data were systematically extracted to present a balanced discussion, ensuring that findings were interpreted within the specific context of brain cancer research.

The gut microbiota and GBA in human health

The human microbiome is a vast aggregate of microorganisms present in different regions of the body comprising viruses, bacteria, archaea, protozoa, and fungi that have evolved along with their hosts over a period of time [70, 71]. The Human Microbiome Project aims to characterise the microbiota across the whole human body and helps to improve the understanding of associations between the microbiome and human health and disease [72, 73]. The healthy gut microbiota can be characterised by high taxa density, microbial gene signatures, and stable functional cores within the microbiome [73, 74]. In order to understand the functional characteristics of the gut microbiome, quantitative analysis of metagenomic, metatranscriptomic, and metaproteomic data revealed the extensive catalogue of 9,879,896 genes from sequenced samples [75]. In addition, it has been reported that microbial signatures unique to individual countries emphasise the influence of environmental factors and host genetics on microbial composition [75]. In fact, each individual possesses a unique core of gut microbiota composition that is colonised during different stages such as birth, infancy, adulthood and old age [76]. Metagenomic analysis and strain-level profiling have demonstrated that the initial colonisers of the gut in vaginally delivered, healthy neonates are maternal faecal bacteria, primarily *Bifidobacterium* and *Bacteroides* [77–81]. Following the completion of breastfeeding, these microbial communities are progressively supplanted by the dominance of *Clostridia* [78]. This pattern has been consistently observed across populations, indicating a biologically regulated process essential to normal human development [82]. In contrast, caesarean section-delivered infants were acquired with *Corynebacteria*, *Staphylococcus*, *Escherichia*, *Propionibacterium spp.*, *Shigella*, *and Bacteroides* from the mother's skin and hospital environment [83]. The gut microbiota composition in adults contained mainly phyla of *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* [83]. In old-age individuals, there has been a greater abundance of *Bacteroides spp.* and *Clostridium* groups [84]. The microbiota present in the gut has numerous roles in almost every aspect of human biology, including absorption of nutrients from the diet, integral maintenance of intestinal barrier, metabolism of cholesterol, bile acids transformation, immune and autoimmune regulation, microbial peptides production, and metabolism of drugs [85–97].

Accumulating evidence suggests that the gut microbiota has a regulatory role in the brain behaviour. Moreover, the gut microbial composition might be associated with other cognitive processes such as mood, learning, stress, and neurodegenerative disorders [98–102]. The GBA is a bidirectional network of communication that links the intestine and brain, including CNS, ANS, ENS, and HPA axis [100, 103]. Mechanistically, the brain modulates the function of the gut by HPA axis, whereas the gut influences the CNS by mediating microbial metabolites, gut hormones, and neuroactive substances through ENS, vagus nerve, circulatory system, and immune system [104–106]. Signalling within the microbiota-GBA can transpire through a diverse array of mechanisms. Metabolites, especially SCFAs, such as acetate, butyrate, propionate, and lactate, produced by the fermentation of carbohydrates, reach the systemic circulation and regulate various biological processes, including epigenetics, gene expression, and immune response [107]. Neurotransmitters such as gamma-aminobutyric acid (GABA), melatonin, serotonin, acetylcholine, catecholamine, and histamine were produced upon modulation of CNS by gut microbiota [108, 109]. Alteration of nutrient availability by gut microbiota leads to the release of molecules from endocrine cells in the enteric region. For instance, galanin, a neuropeptide involved in numerous neurobiological functions, promotes glucocorticoid secretion from the adrenal cortex by stimulating the HPA axis [103, 110, 111]. In short, the gut microbiota influences brain function via the regulation of neurotransmitters and neurotrophic factors, maintenance of intestinal barrier and tight junction integrity, activation of enteric sensory afferents, production of bacterial metabolites, and modulation of mucosal immune responses [22]. Conversely, the brain affects gut microbiota composition and function through alterations in mucus and

biofilm production, modulation of gastrointestinal motility, changes in intestinal permeability, and regulation of immune function [22].

Dysbiosis of the gut microbiota and brain behaviour

A functional and compositional shift in the microbiome that deviates from homeostasis is referred to as gut microbial dysbiosis, with various factors contributing to this, as shown in Fig. 1. This condition disrupts the symbiotic relationship between the host and its microbial community, leading to various health complications [112]. Dysbiosis occurs when this balance is disrupted, favouring pathogenic bacteria over beneficial species, resulting in negative physiological consequences [112]. Gut microbiota dysbiosis can disrupt the components of the GBA, leading to mood, cognition, and behaviour alterations [113]. Stress influences health by altering gut microbiota composition, where the autonomic and circulatory systems, bone marrow-mediated adrenergic signalling, and immune cells transmit psychological stress to the gut [114]. The inflammatory response associated with stress and depression promotes the proliferation of pathogenic bacteria, contributing to dysbiosis and increased intestinal permeability [112]. This compromise in intestinal barrier permeability, often referred to as 'leaky gut', permits bacterial translocation into circulation, triggering systemic inflammation [115, 116]. Both chronic and acute stress, such as marital conflict or laboratory-induced stress, have been shown to compromise intestinal integrity, particularly in individuals with elevated cortisol levels for the latter [117–119]. Moreover, mast cells, along with cortisol, further weaken gut barrier dysfunction, amplifying the inflammatory response [119, 120]. Diet serves as a critical mediator between stress and gut dysbiosis [119]. Even mild stressors can promote unhealthy eating behaviours, while stress-induced alterations in gut microbiota may influence food cravings [119]. Stress and depression not only affect dietary choices but also modulate metabolic responses to food, potentially impacting gut microbial composition. Conversely, gut microbiota alterations may further influence metabolic processes, highlighting the bidirectional relationship between diet, stress, and gut health [119]. It has been proposed that gut dysbiosis and reduced microbial diversity may contribute to dysregulated eating behaviours by favouring the metabolic demands of dominant bacterial species [121]. Mechanistic evidence supports the role of gut microbiota in shaping food choices through multiple pathways. Gut bacteria produce bioactive molecules that can mimic or interfere with human appetite-regulating peptides and hormones [122, 123]. Additionally, they modulate reward pathways by interacting with the appetite-regulating vagus nerve and may influence taste receptor expression [124–126]. Further, microbial-derived neurotransmitters such as serotonin, acetylcholine, and norepinephrine may indirectly affect eating behaviour by altering mood [119, 127]. These findings highlight the complex interplay between psychological stress, gut health, immune function, and associated health consequences.

Dysbiosis in the gut microbiota composition has been implicated in various neuropsychiatric and neurodegenerative disorders, such as depression, anxiety, autism spectrum disorder, Parkinson's disease and Alzheimer's disease [128–133]. Several mechanistic links underlie this connection, primarily involving microbial metabolites, inflammatory responses, vagus nerve signalling, and neurotransmitter modulation. The gut microbiota influences brain function through microbial-derived metabolites such as SCFAs, neurotransmitter precursors, and other bioactive compounds [134]. One of the key pathways connecting gut dysbiosis to brain function is the production of microbial metabolites, particularly SCFAs such as acetate, propionate, and butyrate, which are generated through the fermentation of dietary fibres by commensal bacteria [107, 135–137]. SCFAs exert neuroactive effects by modulating the BBB, influencing microglial activity, and regulating neurotransmitter synthesis [137, 138]. SCFAs can cross the BBB via monocarboxylate transporters on endothelial cells, enhancing BBB integrity by upregulating tight junction proteins [137]. Additionally, SCFAs contribute to the biosynthesis of neurotransmitters such as GABA, serotonin (5-hydroxytryptamine (5-HT)), dopamine, and noradrenaline, thereby affecting neuronal communication and behaviour [137, 139–141]. Butyrate, for instance, plays a crucial role in maintaining the integrity of the BBB and has been shown to exert anti-inflammatory and neuroprotective effects. Mainly, butyrate inhibits the proinflammatory mediators such as interleukin (IL)-1β, IL-8, IL-6 and tumor necrosis factor (TNF)-α and activates the anti-inflammatory cytokine IL-10 [142, 143]. Conversely, a reduction in SCFA-producing bacteria, as observed in dysbiosis, leads to a "leaky gut", facilitating the translocation of bacterial endotoxins such as lipopolysaccharide (LPS) into the systemic circulation [144]. Elevated LPS levels trigger systemic inflammation by activating toll-like receptor (TLR) 4 on immune cells, subsequently inducing the release of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β [145–147]. These inflammatory mediators can cross the BBB and activate microglia, the resident immune cells of the brain, leading to a chronic neuroinflammatory state that is strongly implicated in mood disorders and neurodegenerative conditions [148, 149].

Another significant mechanism linking gut dysbiosis to brain behaviour involves the vagus nerve, a major component of the ANS that provides a direct communication

channel between the gut and the CNS. Certain beneficial gut microbes, such as *Lactobacillus* and *Bifidobacterium*, have been shown to stimulate the vagus nerve, leading to anxiolytic and antidepressant-like effects through the modulation of neurotransmitter release and HPA axis regulation [124, 150]. Conversely, dysbiosis-associated pathogenic bacteria may dampen vagal tone, leading to increased stress responses and altered behavioural outcomes. Moreover, gut microbiota can influence neurotransmitter metabolism either directly by producing neurotransmitters such as GABA, serotonin, and dopamine or indirectly by modulating precursor availability [108, 151]. Approximately 90% of the body's serotonin is produced in the gut by enterochromaffin cells under the influence of gut microbes, with tryptophan metabolism playing a critical role in determining the balance between serotonergic and neurotoxic kynurenine pathway metabolites [152]. Dysbiosis-induced alterations in tryptophan metabolism have been linked to depression, as increased kynurenine levels can enhance neuroinflammation and excitotoxicity via N-methyl-D-aspartate (NMDA) receptor activation [153–155]. Additionally, gut microbes are known to regulate the synthesis of neuropeptides and hormones, including brain-derived neurotrophic factor (BDNF), which plays a vital role in neuronal plasticity, learning, and memory [156]. Reduced BDNF levels have been observed in patients with major depressive disorder and neurodegenerative diseases, and recent studies suggest that probiotic supplementation may help restore BDNF expression by modulating the gut microbiota composition [157]. Another emerging mechanism linking gut dysbiosis to brain behaviour is the role of microbial-derived amyloids and misfolded proteins. Certain gut bacteria, such as *Escherichia coli* and *Bacillus subtilis*, produce amyloid-like proteins that can cross-seed with endogenous amyloid proteins in the brain, potentially contributing to neurodegenerative conditions like Parkinson's and Alzheimer's diseases [158]. This process, known as the "gut-first" hypothesis, suggests that pathological protein aggregates may originate in the gut and propagate to the brain via the vagus nerve or systemic circulation [159, 160]. Furthermore, gut dysbiosis has been shown to influence metabolic pathways by altering bile acid metabolism, lipid homeostasis, and glucose regulation, all of which have implications for brain function [161]. Secondary bile acids produced by gut bacteria have been reported to modulate neuroinflammation and synaptic plasticity through farnesoid X receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5) signalling pathways [162]. Metabolic endotoxaemia, driven by increased intestinal permeability, can also exacerbate insulin resistance, which has been linked to cognitive impairment and Alzheimer's disease pathogenesis [163, 164]. Moreover, recent evidence suggests that the gut microbiota can influence circadian rhythms through microbial metabolite production and host-microbe interactions with the suprachiasmatic nucleus (SCN) of the hypothalamus [165]. Dysbiosis-induced circadian disruptions have been implicated in sleep disturbances, mood disorders, and metabolic dysfunctions [165, 166]. Collectively, these mechanistic insights demonstrate the multifaceted role of gut dysbiosis in shaping brain behaviour and highlight the therapeutic potential of microbiome-targeted interventions, such as probiotics and faecal microbiota transplantation (FMT), in mitigating neuropsychiatric and neurodegenerative conditions. Understanding the precise molecular interactions underlying the GBA will be crucial for developing personalised therapeutic strategies aimed at restoring microbial homeostasis and promoting brain health.

Dysbiosis of gut microbiota in GBM based on randomisation studies

Dysregulation of microorganisms, especially bacteria in the human body, leads to an imbalance in homeostasis, leading to immunosuppression and inflammation and modulating the therapeutic efficiency of drugs [167]. However, the evidence for an association between brain tumour cells and differences in the gut microbial composition has been unexplored. Mendelian randomisation, a crucial analytical method for evaluating the causality of the association between risk factors and pertinent clinical outcomes, proves invaluable in instances where conducting randomised clinical trials is unfeasible due to ethical concerns and associated expenses [168–170]. Studies have used this method to decipher the causal association between gut microbiota and brain cancer and vice-versa (Table 1). For instance, a study utilising the open-source genome-wide association studies (GWAS) summary statistics revealed that nine different taxa were associated with GBM [171]. Amidst them *family Peptostreptococcaceae and genus Eubacterium brachy group* were found to increase the risk, whereas the *family Ruminococcaceae*, *genus Anaerostipes*, *genus Faecalibacterium*, *genus Lachnospiraceae UCG004*, *genus Phascolarctobacterium*, *genus Prevotella7*, and *genus Streptococcus* had a protective role against GBM. After stringent correction, it was found that only the *family Ruminococcaceae* had a protective role against GBM [171]. Another study has reported *Eubacteriumbrachygroup* as a risk factor associated with GBM [172]. Moreover, *Anaerostipes*, *Faecalibacterium*, *Prevotella7 and Ruminococcaceae* were found to have a causal association, exhibiting a protective effect against GBM. It was also reported that *Prevotella7* had a bidirectional causal association with GBM, which could be instrumental in the treatment modalities for GBM [172]. Additionally, another study reported that an increase in the *family Bacteroidaceae and family*

Table 1 Modulation of gut microbiota in brain cancer patients based on findings from randomised studies

Cancer subtype/ Number of patients	Microorganism/metabolites	Effect on glioblastoma	Reference
Glioblastoma patients ($n=91$), Control subjects ($n=218,701$)	*family Ruminococcaceae*, *genus Anaerostipes*, *genus Faecalibacterium*, *genus LachnospiraceaeUCG004*, *genus Phascolarctobacterium*, *genus Prevotella7*, *genus Streptococcus*	↓Risk	[171]
	family Peptostreptococcaceae, *genus Eubacteriumbrachy group*	↑Risk	[171]
Glioblastoma patients ($n=91$), Control subjects ($n=218,701$)	*family Peptostreptococcaceae*, *family Ruminococcaceae*, *family Victivallaceae*, *genus Eubacteriumbrachygroup*, *genus Eubacteriumruminatiumgroup*, *genus Anaerostipes*, *genus Faecalibacterium*, *genus LachnospiraceaeUCG004*, *genus Prevotella7*, *genus RikenellaceaeRC9gutgroup*, *genus Senegalimassilia*	Causal association	[172]
Glioblastoma patients ($n=162$), Control subjects ($n=256,583$)	*family Ruminococcaceae*	↓Risk	[173]
	family Bacteroidaceae, *family Peptococcaceae*, *genus Eubacterium (brachy group)*, *genus Actinomyces*, *genus Bacteroides*, *genus Ruminiclostridium6*	↑Risk	[173]
Glioblastoma patients ($n=91$) Control subjects ($n=174,006$)	*phylum Cyanobacteria*, *family Erysipelotrichaceae*, *family Prevotellaceae*, *genus Eubacterium nodatum group*, *genus Lachnoclostridium*	Protective factors	[174]
	family Rikenellaceae, *family Victivallaceae*, *family Ruminococcus gnavus group*, *family Lactococcus*, *family Ruminococcaceae UCG002*, *family Sellimonas*, *order Desulfovibrionales*	↑Risk	[174]
	Imidazole lactate, N4-acetylcytidine, 1-ribosyl-imidazoleacetate, 1-stearoyl-2-oleoyl-GPE (18:0/18:1), 1-palmitoyl-2-linoleoyl-GPE (16:0/18:2), Androstenediol (3beta,17beta) monosulfate (2), 1-stearoyl-2-linoleoyl-GPE (18:0/18:2), 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4), 1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4), 1-oleoyl-2-arachidonoyl-GPE (18:1/20:4), 1-oleoyl-2-linoleoyl-GPE (18:1/18:2), Pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC), Dihomo-linoleoylcarnitine (C20:2), 1-palmitoyl-2-oleoyl-GPE (16:0/18:1), X-15523	↑Risk	[174]
	Beta-hydroxyisovalerate, 1-palmitoyl-2-oleoyl-GPE (16:0/18:1), X-21607, Decadienedioic acid (C10:2-DC), Retinol (Vitamin A) to oleoyl-linoleoyl-glycerol (18:1–18:2)	↓Risk	[174]
GBM patients (n = 243), Control subjects (n = 287,137)	*family Victivallaceae*, *genus Lactococcus*	↑Risk	[314]
	phylum Cyanobacteria	Protective factor	[314]
Glioma patients ($n=3,301$)	*family Peptostreptococcaceae*, *genus Coprobacter*, *genus Olsenella*	Protective factor	[315]
	family Verrucomicrobia, *family Prevotella7*, *family Euryarchaeota*, *genus Adlercreutzia*, *genus Catenibacterium*	↑Risk	[315]

↑-Increased, ↓-Decreased

Peptococcaceae was correlated with high risk, whereas *family Ruminococcacea* was correlated with a protective effect against GBM. Likewise, increases in the *genus Eubacteriumbrachygroup*, *genus Actinomycetes*, *genus Bacteroides*, *and genus Ruminiclostridium6* were correlated with a high risk of GBM [173]. Another study has reported that reverse Mendelian randomisation analysis has identified a bidirectional causal relationship between the *genus Lactococcus* and the metabolite pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC). This suggests that the occurrence and development of GBM can influence the composition of this bacterial genus and regulate the metabolite levels, highlighting a potential interaction between microbial composition and tumour development [174]. It is worth mentioning that the *family Ruminococcaceae* possesses inflammatory attributes, producing polysaccharides like glucomannan, which have the capability to prime immune cells [171, 175]. It was also reported that *Eubacteriumbrachygroup* could be an ideal biomarker to distinguish colorectal cancer (CRC) samples from healthy samples [172, 176]. Besides, *Anaerostipes* were also reduced in CRC patients compared to healthy controls and had an antitumour effect upon treatment under in vivo conditions [177]. Similarly, *Prevotella7* has shown to be invaluable in prognosis and medical outcomes for CRC patients [178]. Moreover, the *genus Faecalibaterium* has been identified as one of the primary producers of butyrate in the intestine. In vitro studies have demonstrated that butyrate functions as a histone deacetylase (HDAC) inhibitor and an immunosuppressive agent. In addition, butyrate exhibits antitumour properties by inhibiting cancer cells activity and progression [171, 179–181]. These studies suggest an association between gut microbiota composition and the risk of GBM. Further, identifying specific bacterial taxa that may exert a protective effect against

GBM warrants further investigation to develop therapeutic modalities against this disease. However, despite its growing application in causal inference, Mendelian randomisation studies are subject to several inherent limitations that must be critically considered when interpreting their findings. The findings derived from the studies mentioned above were exclusively based on cohorts of European descent and Finnish ancestry, thereby limiting their external validity and precluding the generalisation of these results to ethnically diverse populations [171–174, 314, 315]. Expanding Mendelian randomisation analyses to encompass multi-ancestry cohorts is crucial for enhancing the reliability and generalisability of causal inferences. In addition, these investigations predominantly focused on the GBM subtype of brain cancer, thereby restricting the extrapolation of their findings to other histological subtypes. To enhance the applicability and comprehensiveness of future research, GWAS data encompassing additional brain cancer subtypes, along with the genetic and clinical characteristics of patients, should be incorporated. Further, findings on microbiota candidates are confined to the genus level, lacking resolution at the species or strain level, thereby hindering a more detailed and refined investigation into their relationship with brain cancer. Future microbiota GWAS studies should employ more advanced shotgun metagenomics sequencing analysis pipelines to enhance taxonomic resolution, enabling the identification of microbial associations at the species and strain levels [171–174, 314, 315].

Dysbiosis of gut microbiota in brain cancer patients

The gut microbiota is linked to different subtypes of brain tumours, such as glioma, GBM etc, through the GBA. Identifying diverse gut microbial communities and comparing them with normal samples could offer insights into the diagnosis and prognosis of this disease (Table 2). For instance, a study has reported a difference in the microbial diversity among the healthy controls, gliomas and meningiomas due to the decrease in α-diversity indices (Shannon, Simpson and Chao1) [182]. At the phylum level, there was an increase in *Bacteriodota*, *Proteobacteria*, and *Fusobacteria*, while *Firmicutes* showed a decrease in gliomas and meningiomas compared to healthy controls. Likewise, at the family level, *Bacteriodaceae*, *Prevotellaceae*, and *Acidaminococcaceae* increased while *Lachnospiraceae*, *Ruminococcacea*, and *Selemonadaceae* decreased in the gliomas and meningiomas compared to healthy controls. In addition, at the genus level, *Bacteroides*, *Prevotella*, *Phascolarctobacterium*, *Escherichia/Shigella*, and *Roseburia* increased, while *Agathobacter*, *Lachnospira* and *Parasutterella* decreased in the gliomas and meningiomas compared to healthy controls. Moreover, along with microbial diversity, pathways such as metabolism of D-glutamine and D-glutamate, endocytosis and nucleotide excision repair were decreased in brain tumour patients, especially in the glioma group, in comparison with the meningioma group [182]. Another study has investigated differences in the microbial composition among brain tumour patients, including gliomas, meningiomas, pituitary tumours, brain metastases, and other brain tumour subtypes [183]. It was reported that the alpha diversity of the gut microbiota was lower in the brain tumour patients, as indicated by decreased Shannon, Simpson and Chao1 indices. However, no significant difference was found in the subgroup analysis [183]. The brain tumour group had a higher abundance of phylum-level *Bacteroidetes*, *Fusobacteria*, *and Proteobacteria* and a lower abundance of *Firmicutes* and *Actinobacteria* compared to healthy controls. In addition, *Fusobacteria* and *Proteobacteria* exhibited a lower abundance in the benign brain tumour group comprising of meningiomas and pituitary tumours compared to the malignant brain tumour group comprising of gliomas and brain metastatic brain tumours [183]. Likewise, at the family level, *Bacteroidaceae*, *Enterobacteriaceae* and *Fusobacteriaceae* were higher, and *Lachnospiraceae* and *Akkermanisaceae* were lower in the brain tumours compared to healthy controls. Moreover, the genera *Roseburia* and *Megamonas* were higher, and *Escherichia/Shigella* was lower in benign brain tumours than malignant brain tumours [183]. It was also found that gram-negative, potentially pathogenic, and oxidative stress-tolerant bacteria were abundant and gram-positive were lower in the tumour group [183]. Another study reported that the *Firmicutes* to *Bacteroides* ratio was decreased in IDH-WT and IDH-Mut patients compared to controls [184]. Fascinatingly, *Bacteroidetes*, *Proteobacteria* and *Verrucomicrobia* were increased in the IDH-WT patients but not in IDH-Mut patients compared to controls. Significant increases in the *Akkermansia* and *Akkermansiaceae* were observed in the IDH-WT patients compared to controls but not in IDH-Mut patients [184]. Moreover, another study has reported the change in the composition of gut microbiota in growth hormone-secreting pituitary adenoma and nonfunctional pituitary adenoma characterised by a significant increase in β-diversity compared to controls [185]. *Bacteroides*, *Bifidobacterium*, *Biautia*, *Prevotella*, and *Enterococcus* were increased, and *Escherichia-Shigella* and *Megamonas* were decreased compared to the controls. The serum concentrations of CD4, CD8 and programmed cell death 1 ligand 1 (PD-L1) were higher in growth hormone-secreting pituitary adenoma subtypes than in controls [185]. Subsequently, another study reported the difference in the gut microbial composition of growth hormone-secreting pituitary adenoma patients, which was

Table 2 Modulation of gut microbiota and metabolites in brain cancer patient samples

Brain cancer subtype	Patient samples	Modulation of microbiota/metabolite levels	References
Glioma	Malignant glioma patients ($n=27$), Healthy individuals ($n=41$)	↑*Fusobacterium*, *Akkermansia*, *family Bacteroidaceae*, *family Prevotellaceae*, *genus Bacteroides*, *genus Prevotella* ↓*family Lachnospiraceae*, *family Ruminococcaceae*	[182]
Meningioma	Benign meningioma patients ($n=32$), Healthy individuals ($n=41$)	↑*Enterobacteriaceae*, *family Bacteroidaceae*, *family Prevotellaceae*, *genus Bacteroides*, *genus Prevotella* ↓*family Lachnospiraceae*, *family Ruminococcaceae*	[182]
Glioma	Glioma patients ($n=6$), Controls ($n=6$)	↓5-Hydroxyindoleacetic acid, Norepinephrine	[236]
Brain tumours	Brain tumours (Glioma patients ($n=23$), Meningiomas ($n=32$), Pituitary tumours ($n=24$), brain metastases ($n=13$), others brain tumours ($n=9$)), Healthy controls ($n=57$)	↑*phylum Bacteriodota*, *phylum Fusobacteria*, *phylum Proteobacteria*, *family Bacteroidaceae*, *family Fusobacteriaceae*, *family Enterobacteriaceae*, *genus Bacteroides*, *genus Escherichia/Shigella*, *genus Fusobacterium*, *genus Sutterella*, *Ruminococcus gnavus group* ↓*phylum Firmicutes*, *phylum Actinobacteria*, *phylum Verrucomicrobiota*, *family Bifidobacteriaceae*, *family Barnesiellaceae*, *family RF39*, *family Christensenellaceae*, *family Clostridia_UCG-014*, *family Lachnospiraceae*, *family Monoglobaceae*, *family Ruminococcaceae*, *family Tissierellales*, *family Akkermansiaceae*	[183]
Glioma	Glioma patients (IDH-WT) ($n=39$), Controls ($n=18$)	↑*phylum Bacteroidetes*, *phylum Proteobacteria*, *phylum Verrucomicrobia*, *family Akkermansiaceae*, *genus Akkermansia* ↓*phylum Actinobacteria*, *phylum Epsilonbacteraeota*, *phylum Firmicutes*	[184]
Glioblastoma	Glioblastoma patients ($n=25$), Healthy controls ($n=15$)	↑*phylum Proteobacteria*, *family Bacteroidaceae*, *family Enterobacteriaceae*, *family Alcaligenaceae*, *genus Bacteroides*, *genus Escherichia-Shigella*, *genus Parasutterella*, *Escherichia coli*, *Bacteroides vulgatus*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Clostridium botulinum*, *Prevotella copri*, *Bacteroides uniformis* ↓*phylum Firmicutes*, *family Rikenellaceae*, *family Veillonellaceae*, *family Prevotellaceae*, *genus Prevotella_9*, *genus Ruminococcus_2*, *genus Faecalibacterium*, *Dialister succinatiphilus*, *Ruminococcus flavefacien*, *Bacillus pumilus*, *Faecalibacterium prausnitzii*, *Clostridium leptum*, *Alistipes putredinis*, *Bifidobacterium longum*, *Lactobacillus gasseri*, *Lactobacillus casei*, *Ruminococcus gnavus*	[256]
Craniopharyngioma	Craniopharyngioma patients ($n=15$), Healthy controls ($n=15$)	↑*phylum Bacillota*, *phylum Bacteroidota*, *genus Fusobacterium*, *genus Dorea*, *genus Ruminococcus*, *genus Megamonas*, *genus Clostridium*, *genus Roseburia*, *genus Faecalibacterium*, *Clostridium_sp_AT4*, *Phascolarctobacterium faecium*, *Bacteroides stercoris*, *Roseburia intestinalis*, *Caudoviricetes_sp*, *Romboutsia timonensis*, *Faecalibacterium prausnitzii*, *Dialister succinatiphilus*, *Roseburia inulinivorans* ↑Silibinin, Lysopc 20:0, LysoPC 20:2, Tauroursodeoxycholic acid, JWH 018 N-pentanoic acid metabolite, Xanthosine ↓Ricinine, EMH, Estazolam, (+/−)10(11)-EpDPA, Rifampicin, beta-Nicotinamide mononucleotide	[306]
Brain tumour	Serum samples of brain tumour patients ($n=152$) and healthy controls ($n=198$)	↑Chao1 index, Shannon index, *phylum Firmicutes*, *genus Ruminococcaceae UCG-014*, *genus Lachnospiraceae NK4A136*, *genus Ruminococcaceae UCG-013*, *genus Lactobacillus*, *genus Ruminiclostridium 6*, *genus Peptoclostridium* ↓*phylum Proteobacteria*, *phylum Actinobacteria*, *genus [Eubacterium] coprostanoligenes*, *genus Escherichia-Shigella*, *genus Blautia*, *genus Bifidobacterium*, *genus Streptococcus*, *genus Sphingomonas*	[189]

↑-Increased, ↓-Decreased

characterised by a decrease in the Shannon and Simpson indices compared to the healthy controls [186]. Microflora such as *Oscillibacter*, *Blautia*, and *Rombustia* were increased in the growth hormone-secreting pituitary adenoma patients compared to healthy controls. At the species level, *Odoribacter splanchnicus* and *Alistipes shahii* were increased and *Carnobacterium sp. N15 MGS 207*, *Phascolarctobacterium sp. CAG 207* and *Bacteroides sp. 3_1_19* were decreased in the growth hormone-secreting pituitary adenomas compared to the healthy controls [186]. Also, *Alistipes shahii*, *Odoribacter splanchnicus*, and *Prevotella stercorea* were particularly enriched in the adenomas, while *uncultured phage crAssphage*, *Sutterella wadsworthensis*, and *Sutterella sp. KLE1602* were enriched in the healthy controls. Additionally, the abundance of microbiota was correlated with clinical characteristics of the adenoma patients [186]. *Fusobacterium genus* was positively correlated, and *Lachnospiraceae incertae sedis* and *Oscillibacter* were negatively correlated with biological and radiological remission of the patients. The high abundance of *Enterobacter* was correlated with the higher levels of

preoperative insulin like growth factor (IGF)-1, the ratio of change in IGF-1 index, and the ratio of change in nadir growth hormone in adenoma patients [186]. In addition, another study has reported that discreet microbial communities inhabit tumours that play various roles in physiology [187]. Nejman et al. confirmed the presence of bacteria within seven tumour types, including brain tumours, using a combination of fluorescence in situ hybridisation, immunohistochemistry, culturomics, electron microscopy, and genomic sequencing [187]. Another study has developed an Accu-OptiClearing-based contaminant-free 3D pathology protocol that enables comprehensive visualisation and quantification of bacterial signals across diverse tumour types, facilitating a systematic characterisation of the distribution and morphology of intratumoural bacteria in situ [188]. Furthermore, this study has also established a multi-evidence framework integrating 2D and 3D histological analyses to investigate these signals within human glioma, providing robust validation of the presence of intratumoural bacteria in glioma tissues [188]. Another study has reported the presence of bacterial extracellular vesicles in the brain tissues of brain tumour patients [189]. It has been reported that there was an increased abundance of *genus Bacteroides*, and *genus Erysipelatoclostridium* and a decreased abundance of *phylum Cyanobacteria*, *phylum Saccharibacteria*, *genus Bacteroidales S24-7 group*, *genus Chloroplast (c)*, *genus Lachnospiraceae NK4A136 group*, *genus Prevotella 9*, and *genus Candidatus Saccharimonas* in the tissues of brain tumour patients compared to the controls [189]. Another study has shown that the level of metabolites such as 2-methyl butyl carnitine, N-acetyl putrescine, amino butanal, carnitine, farnesyl diphosphate, shikimate and uridine were significantly higher in the cerebrospinal fluid (CSF) of GBM patients compared to the CSF of controls [190]. It has also been reported that the levels of these metabolites were associated with the mutation profile of the GBM patients. GABA, 2-methylbutylcarnitine, carnitine, deoxycarnitne, propylcarnitne, isobutyryl-L-carnitne, lactate and choline levels were higher in CSF of TP53-WT GBM patients [190]. Cystathionine, nicotinamide, and glycine levels were higher, and lactate, GABA and choline were lower in the CSF of PTEN-WT GBM patients [190]. Higher levels of 2-methylbutyrylcarnitine, acetylcholine, and aminobutanal were associated with better survival of GBM patients compared to GBM patients with low levels of these metabolites. It has also been reported that elevated levels of *Akkermansia muciniphila* might be accountable for the increased levels of shikimate in GBM patients compared to the controls [184, 190, 191]. These studies provide a better understanding that the composition of gut microbiota differs between benign and malignant tumours compared to normal samples (Fig. 2). Moreover, variations in gut microbiota composition were associated with the clinical characteristics of the brain cancer patients. Further comprehensive studies are required to validate these findings, ensuring that alterations in microbial composition can be reliably utilised as a diagnostic biomarker for brain tumours.

Dysbiosis of gut microbiota in model mice

Various reports have associated the development of glioma as a causative factor for dysbiosis in the gut microbial composition of model mice (Table 3). For instance, a study has reported that the development of glioma after the implantation of GL261 cells in mice has affected the microbial composition, resulting in a significant difference in the operational taxonomic units (OTU) compared to faecal samples obtained immediately after implantation [184]. At the phylum level, *Firmicutes* and *Verrucomicrobia* decreased and increased, respectively, and *genus Akkermansia* exhibited an increased abundance after tumour cell implantation [184]. Another study has reported a difference in the β-diversity between control mice and mice implanted with GL261 cells. In addition, *Firmicutes* was decreased, and *Bacteroides* was increased in the tumour mice model [192]. Similarly, another study has reported the difference in the microbial composition between the samples of naïve and glioma mice as demonstrated by increased Shannon and decreased Simpson index [193]. In addition, the relative abundance of genera of bacteria, including *Oscillibacter*, *Anaerotruncus*, *Pseudoflavonifractor*, *Ruminococcus2*, *Intestinimonas*, and *Odoribacter* were increased, and *Coprobacter*, *Anaerofustis*, *Lactobacillus*, and *Barnesiella* were decreased in the glioma compared to naïve mice. It has also been reported that there was a vital change in the *Bacteroidetes* and *Firmicutes* at the phyla levels, and *Barnesiella*, *Coprobacter*, *Lactobacillus*, *Odoribacter*, *Intestinimonas*, *Anaerotruncus*, and *Staphylococcus* at the genus level in the glioma mice during tumour progression [193]. Further, another study has reported a decrease in *Bacteroidetes*, especially *S24-7* and *Actinobacteria* and an increase in *Firmicutes* such as *Clostridia_Clostridiales*, *Clostridiales_Lachnospiraceae*, *and Oscillospira* during the progression of glioma [194]. Moreover, another study has also indicated that the development of glioma resulted in an abnormality in gut microbial composition, brought about by a substantial reduction in β-diversity in model mice compared to control mice [192]. It has also been reported that *Bacteroides* were increased, and *Firmicutes*, *Verrucomicrobiota*, *Proteobacteria*, *Actinobacteria* and *Actinobacteriota* were decreased in the model mice at the phylum level [192]. These studies unequivocally demonstrate that gut microbiota dysbiosis occurs during tumourigenesis. However, the specific role of

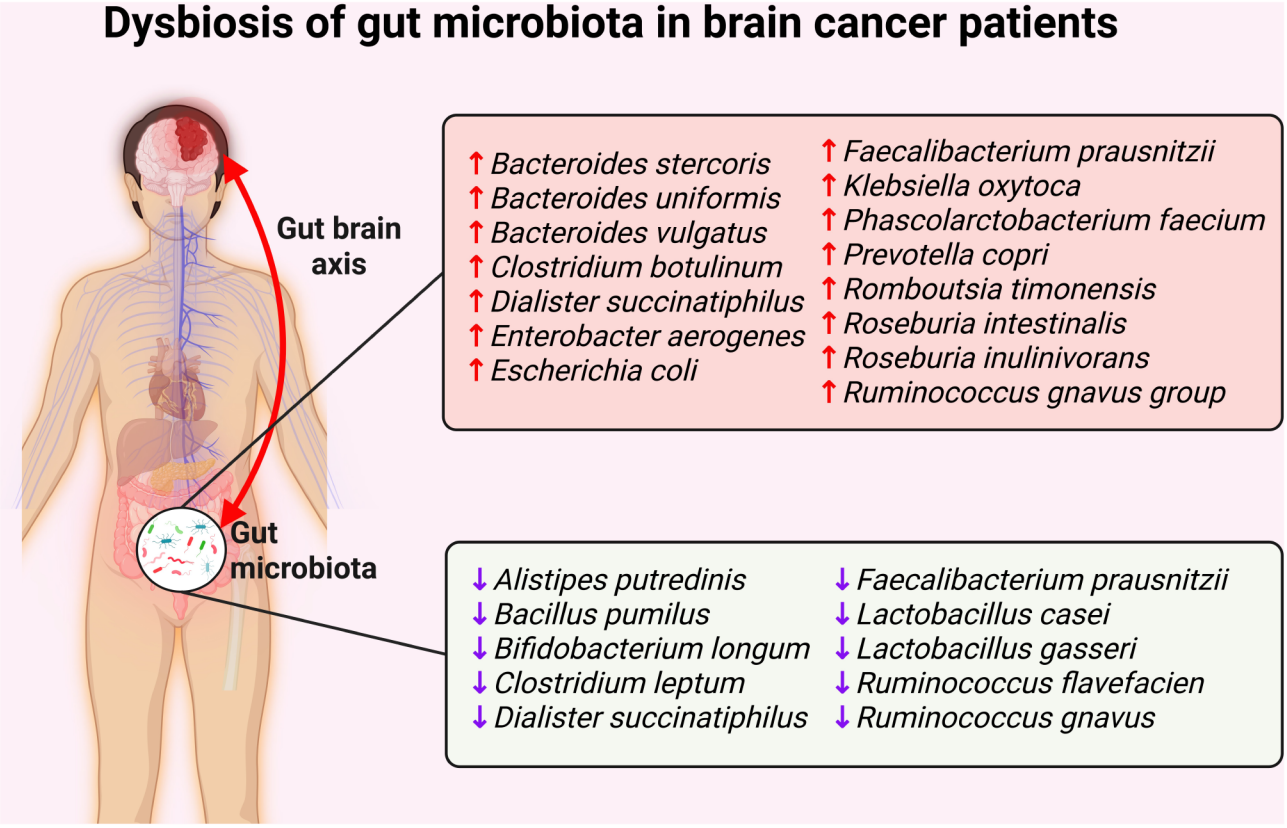

Fig. 2 The modulation of gut microbiota in brain cancer patients and its subtypes [256, 306]. ↑ - increase, ↓ - decrease in the abundance. This figure was created with BioRender.com

these dysregulated microbial communities in the pathogenesis of brain tumours remains elusive and warrants further investigation.

Interaction of gut microbiota with brain cancer and TME

Although evidence suggests that the dysbiosis of gut microbiota has an association with brain cancer, the understanding of the interaction between them has been less clear. The microbe in the gut interacts with the brain cancer cells through various signalling molecules that regulate the tumour progression (Table 4) (Fig. 3). For instance, the GBM mice model (implanted with GL261 and CT-2 A cells) supplemented with high glucose drink of 20% dextrose before tumour cell inoculation for 5 weeks improved the survival of mice when compared to the normal water [195]. However, when a high glucose drink was supplemented to mice lacking gut microbiota, there was no significant difference in the survival between mice groups. 16s rRNA sequencing analysis revealed a significant difference in the microbial composition between mice supplemented for 5 weeks and for 2 weeks and 0 weeks. *Rikenellaceae*, *Desulfovibrionaceae*, *and Odoribacteraceae* were increased, and *Phophyromonadaceae*, *Lactobacillaceae*, *and Lachnospiraceae* were decreased upon high glucose diet supplementation [195]. However, *Desulfovibrionaceae* was increased upon high glucose diet supplementation irrespective of the tumour's existence. Combination of *Desulfovibrionaceae* and high glucose diet supplementation increased the expression levels of immune markers such as programmed cell death 1 (PD-1), cytotoxic T-lymphocyte associated protein 4 (CTLA4) etc., of $CD8^+$ T cells, along with interferon-stimulated genes (Isg). These findings imply that supplementing tumour mice with a high-glucose diet increased the abundance of *Desulfovibrionaceae* in the gut, thereby modulating the antitumour immune response of GBM [195]. Besides, another study also investigated gut microbiota dysbiosis, promoting significant tumour growth in mice upon treatment with antibiotics such as ampicillin, vancomycin, neomycin, and metronidazole [194]. After this treatment, the abundance of antibiotics treated mice were with *Klebsiella*, *Ochrobactrum* and *Enterobacteriaceae*, and untreated mice were with *Bacteroides*, *S24-7*, and *Clostridia_Clostridiales*. In addition, forkhead box P3 (FOXP3) expression was downregulated in the brain tissues of antibiotics-treated mice. However, recovery of gut microbiota upon FMT reversed the dysbiosis of gut microbiota and upregulated FOXP3 expression [194]. Moreover, another study has shown

Table 3 Modulation of gut microbiota and metabolites in murine models of brain cancer

Brain cancer subtype	Models	Modulation of microbiota	Metabolite levels	References
Glioma	Orthotropic xenograft model	↑*Bacteroidota* ↓*Firmicutes*, *Verrucomicrobiota*, *Actinobacteria*, *Actinobacteriota*	↑Polyketide sugar unit biosynthesis, Glycosphingolipid biosynthesis, amino acid metabolism ↓ABC Transporters	[192]
Glioma	C57BL/6 mice (GL261 cells)	↑ *Oscillibacter*, *Anaerotruncus*, *Pseudoflavonifractor*, *Ruminococcus2*, *Intestinimonas*, *Odoribacter*, *Shannon index* ↓ *Coprobacter*, *Anaerofustis*, *Lactobacillus*, *Barnesiella*, *Simpson index*	-	[193]
Glioma	C57BL/6 N mice (GL261 cells)	↑*Verrucomicrobia*, *Bacteriodetes*, *genus Akkermansia* ↓*Firmicutes*	↑Serotonin 3-methyl valerate, caproate, acetylcholine ↓Dihydroxy phenylacetic acid, adenosine, histamine, butyrate, propionate, acetate, norepinephrine, 5-hydroxyindoleacetic acid, GABA, tryptophan, valerate, aspartic acid	[236]
Glioma	C57BL/6 mice (GLI261-Luc cells)	↑*Firmicutes* ↓*Bacteroidia*, *Actinobacteria*	-	[194]
Late-stage Glioma	C57BL/6 mice (GL621 cells)	-	↑Lithocholic acid ↓Reg3g, Il22, Butyrate, Isobutyrate, Propionate, Valerate, Acetate	[196]
Glioma	C57BL/6 mice (GL261 cells)	↑*phylum Verrucomicrobia*, *family Akkermansiaceae*, *genus Akkermansia* ↓*Firmicutes to Bacteroides ratio*, *phylum Firmicutes*	-	[184]

↑-Increased, ↓-Decreased

ABC: ATP binding casette; Reg3g: regenerating family member 3 gamma; Il: interleukin; GABA: gamma-aminobutyric acid;

that gut microbiota depletion with antibiotics improved the survival of the mice bearing tumour cells [196]. It has been found that antibiotic treatment increased the population of $CD45^{low}CD11b^{+}$ and $CD45^{high}CD11b^{+}$ $F4/80^{+}$ cells in the brain of tumour mice [196]. Further, another study has reported that the FMT from growth hormone-secreting and nonfunctional pituitary adenoma patients in immune-reconstructed germ-free mice has increased the tumour volume [185]. It has also been reported that this transplantation increased the serum PD-L1 level, PD-L1 positive cells, and tumour infiltration by $CD8^{+}$ T cells, which was characterised by the increase in the $CD3^{+}CD8^{+}$ population of cells. Microflora such as *Bilophila*, *Lactobacillus*, *Marvinbryantia*, *Alistipes*, *Lachnospiraceae_NK4A136_group*, *Oscillibacter*, *and [Eubacterium]_xylanophilum_group* were decreased and *Anaerostipes*, *Parasutterella*, *[Clostridium]_innocuum_group*, *Hungatella*, *Bacteroides*, *Lachnoclostridium*, *Akkermansia*, *Blautia*, *and Flavonifractor* were increased in the mice transplanted with faecal microbiota from growth hormone-secreting pituitary adenoma patients compared to the controls [185]. These studies suggest that the gut microbiota microenvironment influences the host by modulating metabolic pathways and components of the immune system. However, the precise molecular mechanisms underlying the regulation of these processes remain to be elucidated.

Interaction of gut microbiota with brain cancer and oncotherapy

Chemoresistance poses a significant challenge in the treatment of brain cancers, particularly GBM. This resistance is attributed to factors such as the BBB, intratumoural heterogeneity, and adaptive resistance mechanisms [197–199]. GBM cells employ various strategies to evade chemotherapy-induced cytotoxicity, including the upregulation of drug efflux transporters, activation of survival pathways, and alterations in DNA damage repair mechanisms [197–199]. Additionally, the TME contributes to resistance through hypoxia-induced metabolic reprogramming and immunosuppressive signalling, further complicating therapeutic efforts [197–199]. Temozolomide, an alkylating agent that has been approved and used for treating malignant gliomas. It was developed in the 1980s by the Cancer Research Campaign UK [200, 201]. It has been widely used in treating brain cancer patients since its approval in 1999 and updated in 2023 by the Food and Drug Administration (FDA). It has been used either alone or in combination with radiation and bevacizumab (Avastin®), an anti-angiogenic agent, to improve the patient's survival [202–205]. Several reasons mediate the therapeutic potential of temozolomide, including glioma stem cells, O-6-methylguanine-DNA methyltransferase (MGMT) mutations, DNA repair mechanisms, protein kinase B (Akt) pathway,

Table 4 Mechanistic role of gut microbiota in the regulation of brain cancer

Brain cancer subtype	Cancer patients/models	Intervention/ treatment	Modulation of microbiota	Mechanism of action	References
Glioblastoma	GL261 syngeneic glioblastoma mice	High glucose drink	↑*Rikenellaceae* strain, *Desulfovibrionaceae* strain, *Odoribacteraceae* strain ↓*FR888536_f* strain, *Phophyromonadaceae* strain, *Lactobacillaceae* strain, *Lachnospiraceae strain*	↑$CD4^+$ T_C cell/$FOXP3^+$ T_{reg} ratio, $CD8^+$ T_C cell/$FOXP3^+$ T_{reg} ratio, IFNγ, Isg15 Ifi27l2a, Ifit1, Ifit2, Ifit3 ↓*Isg20*, *Ifitm1*, *Ifitm2*, and *Ifitm3*	[195]
	GL261 syngeneic glioblastoma mice	High glucose drink + *Desulfovibrio vulgaris*	↑*Desulfovibrio vulgaris*	↑Survival	[195]
Glioma	U87-MG, U251-MG cell lines	Serum from Taohong Siwu Decoction fed SPF mice	-	↑CSF3, PLAU, UHRF1, FOSL1, IL1B, DUSP6, MCM2, CCND1, WNT7B, MCM5, MCL1, SH2B3, MCM3, HNRNPM, PALM2AKAP2 ↓Proliferation, Colony formation, ATF4, FDFT1, ADCY8, CYP1B1, EREG, FAM114A1, EIF4A2, AKR1B1, C14orf132, COL1A2, CDKN1A, ASPH, DEPP1, AKR1C1, DDIT4	[253]
Glioma	Orthotropic xenograft model	Temozolomide	↑*α-diversity indices of Chao1*, *Shannon*, *Simpson*, *Firmicutes*, *Verrucomicrobiota* ↓*Bacteroidota*	-	[192]
	Luci-GL261 glioma orthotropic xenograft model	Temozolomide	↑*Alloprevotella*, *Desulfovibrio*, *Muribaculum* ↓*Akkermansia*	↓IL-1β, TNF-α, %Macrophages, %Cytotxic T lymphocytes	[192]
Glioma	C57BL/6 mice (GL261 cells)	*Bifidobacterium lactis* + *Lactobacillus plantarum*	↑*Firmicutes/Bacteroidetes ratio*, *Bifidobacterium lactis*, *Lactobacillus plantarum*, *Firmicutes* ↓*Bacteriodetes*,	↑Survival, Neurobehaviour, Occludin, PTEN, threonic acid, conduritol b epoxide 1, ascorbate, 5-alpha-cholestan-3-one 1, maltose, 2′-deoxyadenosine, succinate semialdehyde 1, dehydroascorbic acid 1, 4-hydroxypyridine, L-dopa 1 ↓Tumour volume, $Ki67^+$ cells/section, Survivin, p-PI3K, N-cadherin, L-kynurenine 1, beta-glutamic acid 1, albendazole 1, coprostan-3-one, citraconic acid 3, glucosaminic acid, galactinol, methyl yellow, elaidic acid, D-Arabitol	[272]

Table 4 (continued)

Brain cancer subtype	Cancer patients/models	Intervention/ treatment	Modulation of microbiota	Mechanism of action	References
Glioma	C57BL/6 mice (GL261 cells)	Temozolomide	↑*Intestimonas*, *Anaerotruncus*, *phylum Verrucomicrobia*, *phylum Deferribateres*, *genus Akkermansia*, *genus Bifidobacterium*, *genus Coprobacillus*, *genus Clostridium_XVIII*, *genus Sporobacter*, *genus Romboutsia*, *genus Bilophila*, *genus Anaerotruncus*, *genus Vampirovibrio*, *genus Mucispirillum*, *genus Oscillibacter* ↓*genus Coprobacter*, *genus Barnesiella*	↑Glutathione metabolism, fatty acid biosynthesis, mineral absorption, cofactor- and vitamin- biosynthesis	[193]
Glioma	C57BL/6 N mice	ABX (Vancomycin + Gentamycin + Sucralose)	↑*family Alcaligenaceae*, *family Burkholderiaceae* ↓*family Prevotellaceae*, *family Rikenellacaea*, *family Helicobacteraceae*	↑Tumour volume, $CD27^+/CD11b^-$ NK cells, ARG1, P2RY12, iNOS mRNA ↓Total NK cells number, Shannon index, $CD27^+/CD11b^+$ NK cells	[216]
Glioma	C57BL/6 mice (GLI261-Luc cells)	Ampicillin, Vancomycin, Neomycin, Metronidazole	↑*Ochrobactrum*, *Klebsiella*, *Enterobacteriaceae* ↓*Bacteroides*, *Firmicutes*	↑Tumour Volume ↓FOXP3	[194]
	C57BL/6 mice (GLI261-Luc cells)	Ampicillin, Vancomycin, Neomycin, Metronidazole + Faecal microbiota transplantation	↑*genus Bacteroides*, *genus S24-7*, *genus Parabacteroides*, *phylum Bacteriodetes* ↓*phylum Proteobacteria*, *phylum Cyanobacteria*	↓Tumour Volume ↑FOXP3	[194]
Glioma	Glioma patient (IDH-WT) samples	Temozolomide	↓*phylum Verrucomicrobia*, *Akkermansiaceae*, *Akkermansia*	-	[184]
Malignant glioma	Recurrent Malignant Glioma patients ($n = 15$)	Bevacizumab + Temozolomide	↑*phylum Actinobacteria*, *phylum Firmicutes*, *genus Fusicatenibacter*, *genus Roseburia*, *genus Blautia*, *genus Ruminococcus*, *genus Anaerotruncus*, *genus Clostridium_IV*, *genus Anaerostipes*, *genus Erysipelotrichaceae_incertae_sedis*, *genus Sutterellaceae*, *genus Parasutterella*, *genus Erysipelotrichia*, *genus Erysipelotrichales*, *genus Erysipelotrichaceae*, *genus Eggerthella*, *genus Intestinimonas*, *genus Coriobacteriaceae*, *genus Coriobacteriales*, *genus Dorea*, *genus Faecalibacterium*, *genus Ruminococcaceae*, *genus Escherichia_Shigella*, *genus Clostridium_XlVa*, *genus Lachnospiraceae*, *genus Clostridiales*, *genus Clostridia* ↓*phylum Bacteroidetes*, *phylum Cyanobacteria*, *genus Streptophyta*, *genus Bacteroides*, *genus Scardovia*, *genus Veillonella*	-	[207]

Table 4 (continued)

Brain cancer subtype	Cancer patients/models	Intervention/ treatment	Modulation of microbiota	Mechanism of action	References
Glioma	C57BL/6 mice (GL261 cells)	B. breve, B. longum, B. lactis, and B. bifidum in sterile saline water (4×10^9 CFU/0.4 ml)	↑*phylum Firmicutes*, *phylum Bacteroidetes*, *phylum Cyanobacteria*, *phylum Actinobacteria*, *genus Bifidobacterium*, *genus Achromobacter*, *genus Anaerococcus*, *genus Bacillus*, *genus Enterobacter*, *genus Citrobacter*, *genus Escherichia/Shigella*, *genus Enhydrobacter*, *genus Acinetobacter*, *genus Wautersiell*, ↓*phylum Proteobacteria*, *phylum Myxococcota*, *genus Raoultella*, *genus Hydrogenophilus*	↑Medial survival, Chao1 index, Shannon index, Simpson index, aerobic respiration I (cytochrome c), fatty acid salvage pathway, 1-amino-propan-2-ol, tyrosine, 4-methylene-2-pyrrolidinecarboxylic acid ↓Tumour volume, phosphocholine, 1,2,3,4-tetrahydroxybutane levels in serum, p-MEK, p-ERK1/2, Wnt5a, PD-L1, NF-κB	[273]
Glioma	C57BL/6 N mice (GL261 cells)	ABX (Vancomycin, Gentamicin) and sucralose (0.5%)	-	↑Tumour volume, $CD34^+$ vessel-like structures, Vasculogenesis, CD31, VEGFα, MMP9, CD68, $CD133^+CD34^+$ cells, Gly-derivative, leucine, valine, lactate ↓IL1β, TNFα, ARG1, CD206, speed of process movement of microglial cells, butyrate, propionate, acetate, hypoxanthine, beta-xylose, trimethylamine, uracil, alpha-glucose, nicotinate, beta-galactose, propionate, bile salts 1, glutamate	[237]
	C57BL/6 N mice (CT-2 A cells)	ABX (Vancomycin, Gentamicin) and sucralose (0.5%)	-	↑Tumour volume, Tumour vasculogenesis	[237]
Malignant gliomas	C57BL/6 mice (GL261 cells)	Delta-24-RGDOX	↑*Actinobacteria*, *Verrucomicrobia*, *Bifidobacterium*, *Akkermansia* ↓*Firmicutes/Bacteroidetes* ratio	↓Chao1 index, Shannon index	[309]
	C57BL/6 mice (GL261 cells)	Indoximod	↑*Turicibacter* ↓*Firmicutes/Bacteroidetes* ratio	↑Survival ↓Chao1 index, Shannon index	[309]
Glioma	BALB/C nude mice (U251 cells)	*Schizophyllum commune* fruiting body polysaccharides	↑number of OTUs, *genus Akkermansia*, *genus unclassified_Oscillospiraceae*, *genus Parabacteroides*, *genus [Eubacterium]_fissicatena_group*, *unclassified_Lachnospiraceae*, *Akkermansia muciniphila*, *Ligilactobacillus murinus*, *unclassified_Oscillospiraceae*, *Parabacteroides goldsteinii*, *Phocaeicola vulgatus* ↓*phylum Verrucomicrobiota*, *phylum Bacteroidetes*, *genus Lachnospiraceae_NK4A136_group*, *genus Ligilactobacillus*	↑E-cadherin, cleaved-Caspase-3, ARH1 ↓Tumour volume, N-cadherin, Bcl-2, p-PI3K, p-Akt	[301]

Table 4 (continued)

Brain cancer subtype	Cancer patients/models	Intervention/ treatment	Modulation of microbiota	Mechanism of action	References
Glioma	C57BL/6J mice (GL261 cells)	RO water supplemented with Ampicillin, Neomycin, Metronidazole, Vancomycin	↑*phylum Proteobacteria*, *genus Morganella*, *genus Parasutterella* ↓OTU counts, ACE index, Shannon index, Chao index, *phylum Firmicutes*, *phylum Bacteroidetes*, *phylum*, *Tenericutes*, *phylum Actinobacteria*, *phylum Deferribacteres*, *phylum Candidatus_Saccharibacteria*, *genus Lactobacillus*, *genus Prevotella*, *genus Desulfovibrio*	↑Tumour volume, IL-10, % of $CD206^+$ TAMs ↓Survival, IFN-γ, IL-2, TNF-α, % of $CD86^+$ TAMs, $CD86^+/CD206^+$ ratio, propionic acid, butyric acid, valeric acid, caproic acid, acetic acid (in serum)	[217]
Glioma	C57BL/6J mice (GL261 cells)	RO water supplemented with Ampicillin, Neomycin, Metronidazole, Vancomycin + Faecal microbiota transplantation (from normal mice)	-	↑Survival ↓Tumour volume	[217]
Glioma	C57BL/6J mice (GL261 cells)	RO water supplemented with Ampicillin, Neomycin, Metronidazole, Vancomycin + SCFAs (propionic acid + butyric acid + valeric acid)	-	↑Survival, % of TAMs, $CD86^+/CD206^+$ ratio, % of $CD86^+$ TAMs, CD86, MHC-II, iNOS/Arg-1 ratio, GLUT1, HIF-1α, HK2, PKM2, LDHA ↓Tumour volume, $CD206^+$ TAMs, CD206	[217]
Optic pathway glioma	$Nf1^{OPG}$ mice (germ free)	-	-	↑RNFL thickness ↓%$Ki67^+$ cells, %$Blbp^+$ cells, %$Olig2^+$ cells, Ccl4, Ccl5, $CD8^+$ cells, Ccl3, TGFβ	[283]
	$Nf1^{OPG}$ mice	Vancomycin	-	↑RNFL thickness ↓%$Ki67^+$ cells, %$Blbp^+$ cells, %$Olig2^+$ cells, Ccl4, Ccl5, CD8a, $CD8^+$ cells, Ccl3, TGFβ	[283]
	$Nf1^{OPG}$ mice (germ free)	Faecal microbiota transplantation (from $Nf1^{OPG}$ mice)	-	↑%$Ki67^+$ cells, %$Blbp^+$ cells	[283]
	$Nf1^{OPG}$ mice	Vancomycin + *Bacteroides*	-	↑%$Ki67^+$ cells, %$Blbp^+$ cells ↓RNFL thickness	[283]

↑-Increased, ↓-Decreased

AKR1B1: aldo-keto reductase family 1 member B; ARG1: arginase 1; ADCY8: adenylate cyclase 8; AKR1C1: aldo-keto reductase family 1 member C1; ARH: ADP-ribosylarginine hydrolase; ASPH: aspartate beta-hydroxylase; ATF4: activating transcription factor 4; Bcl-2: BCL2 apoptosis regulator; C14orf132: mitogen-activated protein kinase 1 interacting protein 1 like; Ccl: C-C motif chemokine ligand; CCND1: cyclin D1; CDKN1A: cyclin dependent kinase inhibitor 1 A; COL1A2: collagen type I alpha 2 chain; CSF3: colony stimulating factor 3; CYP1B1: cytochrome P450 family 1 subfamily B member 1; DDIT4: DNA damage inducible transcript 4; DEPP1: DEPP autophagy regulator 1; DUSP6: dual specificity phosphatase 6; EIF4A2: eukaryotic translation initiation factor 4A2; EREG: epiregulin; ERK: extracellular related kinase; FAM114A1: family with sequence similarity 114 member A1; FDFT1: farnesyl-diphosphate farnesyltransferase 1; FOSL1: FOS like 1, AP-1 transcription factor subunit; FOXP3: forkhead box P3; HIF-1: hypoxia inducible factor 1; HK2: hexokinase 2; HNRNPM: heterogeneous nuclear ribonucleoprotein M; Ifi: interferon alpha inducible protein; Ifit: interferon-induced protein with tetratricopeptide repeats; IL: interleukin; iNOS: inducible nitric oxide synthase; Isg: interferon-stimulated genes; LDHA: lactate dehydrogenase A; MCM: minichromosome maintenance complex component; MCL1: MCL1 apoptosis regulator; MEK: mitogen-activated protein kinase kinase; MMP: matrix metallopeptidase; NF-κB: nuclear factor kappa B subunit; P2RY12: purinergic receptor P2Y12; PALM2AKAP2: paralemmin 2 and A-kinase anchoring protein 2 fusion; PD-L1: programmed cell death 1 ligand 1; PI3K - phosphatidylinositol-4,5-bisphosphate 3-kinase; PKM2: pyruvate kinase M1/2; PLAU: plasminogen activator, urokinase; PTEN: phosphatase and tensin homolog; SH2B3: SH2B adaptor protein 3; TAMs: tumour associated macrophages; T_c cell: cytotoxic T cell; TGFβ: transforming growth factor beta; TNF: tumor necrosis factor; T_{reg}: regulatory T cell; UHRF1: ubiquitin like with PHD and ring finger domains 1; VEGF: vascular endothelial growth factor; Wnt: wingless-type MMTV integration site family; Wnt5a: Wnt family member 5a; WNT7B: Wnt family member 7B

Wnt/β-Catenin pathway, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, autophagy, epigenetic modifications etc., as discussed elsewhere [206]. In line with this, a study has shown that temozolomide efficacy in mice-bearing tumour cells was associated with microbial composition in the gut [192]. Temozolomide treatment exhibited a diverse antitumour effect in mice, significantly reducing tumour size in one

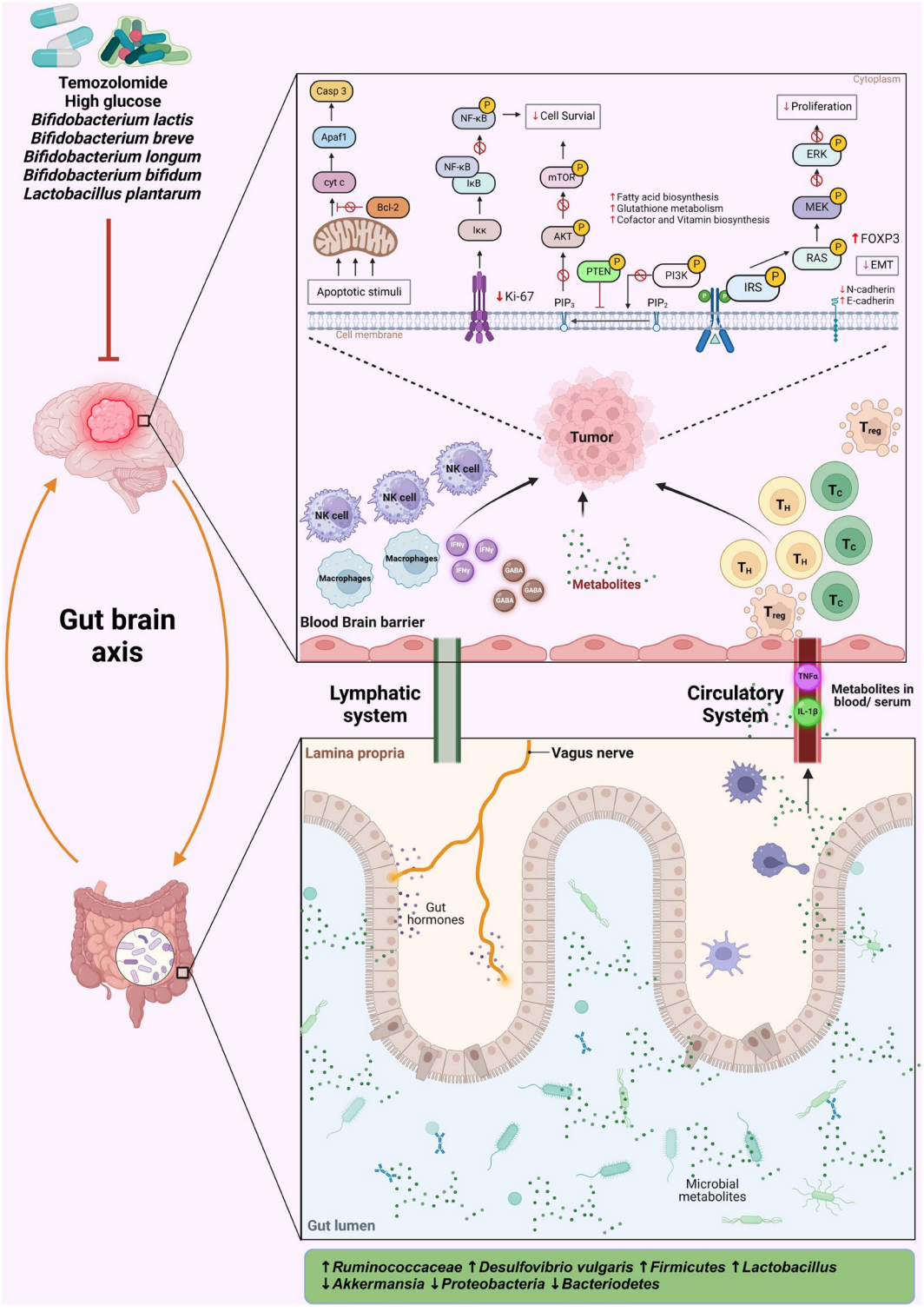

Fig. 3 Impact of the therapeutic regimen administration on gut microbiota and microbial metabolites in brain cancer therapy: Administration of temozolomide, bevacizumab, high glucose diet, probiotics such as *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium bifidum and Lactobacillus plantarum* changes the dysbiosis in the gut microbial composition. The microbial metabolites produced by the gut microbiota pass through the lymphatic, circulatory system, etc., reaching and crossing the blood-brain barrier and modulating the components in the microenvironment, ultimately regulating the progression of brain cancer. ↑ - increase, ↓ - decrease in the abundance. This figure was created with BioRender.com

group while showing no alteration in another, indicating the presence of a cell subpopulation with resistance activity. Gut microbiota composition was significantly varied between the sensitive and non-sensitive groups, demonstrated by significant differences in β-diversity index. Further analysis revealed that *Bacteroides*, *Alloprevotella*, *Desulfovibrio*, and *Muribaculum* were increased, and *Akkermansia* was decreased in the non-sensitive mice [192]. Another study has reported the modulation in the gut microbial composition upon temozolomide treatment in glioma mice [193]. At the phylum level, *Verrucomicrobia* was increased, and at the genus level, *Anaerotruncus*, *Akkermansia*, *Bifidobacterium*, *Coprobacillus*, *Intestimonas*, *and Clostridium_XVIII* were increased, and *Coprobacter* was decreased in temozolomide treated mice when compared to the vehicle-treated mice. This increase in gut microbial composition may represent an innate anti-tumour response occurring during tumourigenesis and play a significant role in the host's anti-tumour defence mechanisms by regulating various metabolic pathways [193].

Further, analysis of stool samples from glioma patients either possessing IDH-WT or IDH-Mut phenotype treated with temozolomide exhibited a non-significant decrease in the abundance of *phyla Verrucomicrobia*, *phyla Akkermansiaceae*, *and genus Akkermansia* [184]. Furthermore, another study has investigated the gut microbial composition in recurrent malignant glioma patients treated with a combination of bevacizumab and temozolomide [207]. The levels of these genera *Anaerostipes*, *Anaerotruncus*, *Blautia*, *Clostridia*, *Clostridiales*, *Clostridium_IV*, *Clostridium_XlVa*, *Coriobacteriaceae*, *Coriobacteriales*, *Dorea*, *Eggerthella*, *Erysipelotrichaceae*, *Erysipelotrichaceae_incertae_sedis*, *Erysipelotrichales*, *Erysipelotrichia. Escherichia_Shigella*, *Faecalibacterium*, *Fusicatenibacter*, *Intestinimonas*, *Lachnospiraceae*, *Parasutterella*, *Roseburia*, *Ruminococcaceae*, *Ruminococcus*, *and Sutterellaceae* were lower in the group administered with bevacizumab and temozolomide when compared to the group administered with temozolomide alone [207].

In addition, other therapeutic agents also have a role in regulating the intestinal microbiota. A case study has been reported that administration of the neurotrophic tyrosine receptor kinase (NTRK) inhibitor Larotrectinib as a second-line therapy to a 66-year-old man for a month with high-grade glioma resulted in the remodelling of both oral and gut bacterial communities [208]. This treatment notably reduced the genera *Alistipes*, *Anaerostipes*, *Bifidobacterium*, *Blautia*, *Dorea*, *Faecalibacillus*, and *Mediterraneibacter* and increased *Clostridium*, *Fusicatenibacter*, *Merdicola*, and *Romboutsia*. At the species level, there was an increase in the abundance of *Intestinibacter sp900540355*, *Phocaeicola plebeius*, *Pseudoruminococcus massiliensis*, *Rikenella microfusus*, *Barnesiella intestinihominis*, *CAG-452 sp000434035*, *Bacteroides fragilis*, *Turicibacter sanguinis*, *and Alistipes communis* and decrease in the *Bariatricus comes*, *Faecalibacterium prausnitzii*, *Intestinimonas butyriciproducens*, *Parvimonas sp001553085*, *Gemella morbillorum and Finegoldia magna* [208]. Among these, species belonging to the *Streptococcus* genus were enormously increased upon Larotrectinib treatment, whereas *Fusobacterium vincentii*, *Mogibacterium timidum*, *Prevotella oris*, and *Eggerthia catenaformis* were drastically reduced in the oral microbiota [208]. However, the mechanistic role of this modulation in microbial communities has not been reported and has to be further elucidated with in vitro or in vivo experiments [208]. The findings from the aforementioned studies strongly indicate that the composition of the gut microbiota plays a crucial role in modulating the therapeutic efficacy of chemotherapy across various brain cancer subtypes. Inclusively, the distinct microbial communities present in the gut can influence drug metabolism, immune responses, and TME dynamics, thereby impacting treatment outcomes.

Gut microbiota-mediated immunomodulation in the brain cancer microenvironment

The TME of GBM is highly heterogenous and dynamic, containing a series of non-neoplastic cells such as astrocytes, endothelial cells, pericytes, immune cells including CD4 T cells, CD8 T cells, T_{reg} cells, TAMs, etc [209–214]. It is also classified as a cold tumour because of the consistent failures of immunotherapies targeting PD-L1 and CTLA4 in GBM [215]. Given the immunosuppressive nature of the GBM microenvironment, recent studies have explored the role of the gut microbiota in modulating systemic and local immune responses. The gut microbiota plays a crucial role in shaping these responses, influencing the TME even in distant organs such as the brain. Emerging evidence suggests that gut dysbiosis can alter immune cell recruitment, cytokine signalling, and metabolic pathways, thereby modulating brain cancer progression. Understanding the mechanisms by which gut microbes influence immune regulation in the brain cancer microenvironment could provide novel therapeutic insights, particularly in overcoming the limitations of current immunotherapies. For instance, a study has reported that modulation of the microbiota using antibiotics can regulate innate immunity by altering the population of NK cells in tumour model mice [216]. Also, there was a non-tumour specific reduction of $CD27^+$/$CD11b^+$ and an increase of $CD27^+$/$CD11b^-$, a subset of NK cells, in the tumoural and non-tumoural hemisphere, bone marrow, and spleen of the model mice [216]. However, recovery of gut microbiota by interrupting the treatment of antibiotics reversed these effects. Antibiotic treatment of mice increased the *Alcaligenaceae* and

Burkholderiaceae families and decreased the *Rikenellacaea*, *Prevotellaceae*, *and Helicobacteraceae* families [216]. In addition, arginase 1 (ARG1), inducible nitric oxide synthase (iNOS) mRNA and purinergic receptor P2Y12 (P2RY12) expression were increased in the $CD11b^+$ microglial cells of the brain of antibiotics-treated mice [216]. In addition, another study has evaluated the impact of gut microbiota dysbiosis on the modulation of the immune microenvironment in glioma [217]. The gut microbiota dysbiosis induced by the antibiotic supplementation resulted in decreased mRNA levels and tissue content of interferon gamma (IFN-γ), IL-2 and TNF-α and increased IL-10, a pro-tumour-related cytokine [217]. It has also been reported that the antibiotic treatment induces a shift towards immunosuppressive TME and promotes polarisation of M2 phenotypes characterised by decreased $CD86^+/CD206^+$ ratio, percentage of $CD86^+$ TAMs and increase in the percentage of $CD206^+$ TAMs [217]. Moreover, a study has evaluated the role of gut microbiota in the immune modulation of TME in the presence of temozolomide [192]. This study reported that serum concentrations of IL-1β and TNF-α were increased along with macrophage (F4/80) and cytotoxic T lymphocytes (CD8α) cells percentage in sensitive mice compared to mice non-sensitive to temozolomide. It has been previously reported that IL-1β and TNF-α are one of the important mediators between the gut microbiota and the host inflammatory responses [218, 219]. However, these effects were reversed in the absence of gut microbiota when a cocktail of broad-spectrum antibiotics was administered along with temozolomide. These findings suggest that the presence of the distinct microbiota can counteract the immunosuppressive TME in sensitive mice [192]. Another study has evaluated the role of gut microbiota in the anti-tumour immune response of GBM [195]. High glucose supplementation to NOD/Shi-SCID, IL-2Rγnull (NOG) mice implanted with GL261 cells did not improve the survival compared to control drink. In addition, $CD8^+$ T cell/$FOXP3^+$ Treg and $CD4^+$ T cell/$FOXP3^+$ Treg ratios were increased in the high glucose drink group compared to the controls [195]. Isgs such as Isg15, interferon alpha inducible protein (Ifi)27l2a, interferon-induced protein with tetratricopeptide repeats (Ifit)1, Ifit2 and Ifit3 were increased, and Isg20, interferon induced transmembrane protein (Ifitm)1, Ifitm2, Ifitm3 were decreased in the $CD8^+$ T cells of high glucose drink group. Besides, the bacteria from the family *Desulfovibrionaceae* have regulated the anti-tumour response, particularly through $CD107a^+NKG2D^+CD4^+$ T cells [195]. Moreover, administration of the anti-PD-1 along with high glucose drink supplementation increased the ratio of $CD4^+$ T cells to $Foxp3^+$ Treg cells. Also, the expression of the interferon-stimulated genes such as Isg15, Ifi27l2a, and Isg20 was increased in $CD8^+$ T cells and $CD4^+$ T cells [195]. These findings indicate that modulating the gut microbiota through supplementation with a high-glucose drink may serve as an adjuvant to enhance the efficacy of immune checkpoint inhibitors [195]. However, the precise mechanistic role of the family *Desulfovibrionaceae* in regulating these immune cells requires further elucidation to achieve a comprehensive understanding of this process.

Recently, immunotherapy with immune checkpoint inhibitors has attained a significant milestone in the realm of cancer therapeutics, especially in brain cancer [220–223]. While preclinical studies have demonstrated the safety and efficacy of anti-PD-1 treatment, a substantial proportion of patients fail to respond favourably to this therapy [220, 224–226]. This highlights the imperative need for further research to elucidate the factors contributing to therapeutic resistance and to develop alternative and combinatory strategies to enhance its effectiveness in clinical trials. Addressing the challenge of non-responsiveness is crucial to optimise patient outcomes and maximise the potential benefits of anti-PD-1 treatment. Interestingly, in accordance with this, a study has reported that anti-PD-1 treatment has improved the survival of the mice bearing GL261 cells with humanised microbiome [227]. These responders have exhibited an increase in the level of $CD8^+(IFN\gamma^+)$ T cells, $CD4^+(IFN\gamma^+)$ T cells and $CD8^+$/Treg ratio compared to the non-responders of anti-PD-1 treatment. *Bacteroides cellulosilyticus* and *Blautia producta* were higher in the respondents than in the non-responders. Also, *Alistipes indistinctus*, *Blautia hydrogenotrophica*, *and Eubacterium limosum* were unique to responders compared to the non-responder group [227]. Another study has identified *Desulfovibrio vulgaris* as a key modulator of T-cell mediated anti-tumour immune responses in the GBM mice model [195]. Treatment with *D.vulgaris* enhanced the synergistic efficacy of anti-PD-1 therapy by increasing the survival time of model mice compared to mice when treated with anti-PD-1 therapy alone [195]. These studies suggest that the gut microbiota plays a pivotal regulatory role in the progression of brain tumours by modulating the immune microenvironment, either by promoting or suppressing the activity of immune cells. Moreover, the presence of distinct microbial communities within the gut influences the efficacy of immunotherapeutic interventions in brain cancer and its subtypes. Variations in gut microbial composition may contribute to differential responses to immune-based therapies, highlighting the potential for microbiota-targeted strategies to enhance therapeutic outcomes in brain malignancies.

Interaction of gut microbiota with brain cancer through metabolites

Metabolites are diminutive molecules implicated in cell signalling, furnishing the substrates necessary for metabolism, macromolecular synthesis, and signal transduction. These metabolites not only furnish the molecules to the cell but also regulate various signalling cascades, contributing to numerous metabolic disorders and cancer [228–232]. Interestingly, abundant microbiota residing in the intestine can generate copious metabolites that influence the regulation of homeostasis and cancer [233–235]. Gut microbiota dysbiosis disrupts host metabolic homeostasis, altering metabolite profiles and dysregulated metabolic pathways. For instance, a study has reported that the metabolite levels were modulated in the mice upon tumourigenesis compared to control mice. It was observed that metabolites such as dihydroxy phenylacetic acid, butyrate, adenosine, propionate, histamine, norepinephrine, acetate, GABA, 5-hydroxy indole acetic acid, valerate, tryptophan, and aspartic acid were decreased and caproate, serotonin 3-methyl valerate, and acetylcholine were increased upon tumour growth [236]. Another study has revealed that late-stage glioma was associated with anomalous changes in the level of metabolites in mice implanted with tumour cells [196]. It was reported that Reg3g and Il22 were decreased in the ileum and jejunum of the mice's colon. In addition, the amount of SCFAs such as butyrate, propionate, isobutyrate, and valerate were decreased, and bile acids such as lithocholic acid were increased in the cecum of the tumour mice [196]. Administration of these caecal metabolites from glioma mice to mice treated with antibiotics such as amphotericin B, metronidazole, neomycin, ampicillin, and vancomycin had worse survival than those received from healthy mice [196]. Similarly, another study has investigated the potential of gut-derived metabolites in modulating the bidirectional crosstalk between neuronal and glial cells within the glioma microenvironment [237]. Antibiotic treatment to a glioma mice model induced dysbiosis of the gut microbiota, leading to increased and decreased expression of proangiogenic and inflammatory genes compared to the control mice. This treatment enhanced the vasculogenesis in a glioma stem cell-dependent manner as evidenced by the elevated expression of $CD34^{+}CD31^{+}$ vessel-like structures and an increased abundance of $CD133^{+}CD34^{+}$ cells within the tumour core and also facilitated the glioma cells to transdifferentiate into endothelial phenotype [237]. Metabolomic profiling has elucidated the association of glycine and its derivatives with antibiotic treatment-induced tumour progression in glioma. Treatment of glioma, microglia, and murine endothelial cells with glycine induced the acquisition of stem-like properties and a pro-angiogenic phenotype within the TME [237]. In addition, inhibition of glycine transporter 1 further facilitated tumour progression and enhanced vasculogenesis in murine models [237].

Certain metabolites produced by specific microbial strains exert beneficial effects like anti-tumour effects on the host by modulating physiological processes, including immune regulation and metabolic homeostasis. These bioactive compounds might contribute to maintaining host health and may have therapeutic potential in disease management. For instance, Ahmed S et al. have characterised and screened the bacterial metabolites' neuroprotective ability to decrease the secretion of proinflammatory IL-6 in GBM astrocytoma cells [238]. The U373 cells were exposed to a panel of 50 bacterial strains' cell-free supernatants, among which *Parabacteroides distasonis* (MRx0005) and *Megasphaera massiliensis* (MRx0029) demonstrated significant efficacy in attenuating IL-6 secretion following LPS stimulation. These strains were initially isolated from faecal samples obtained from healthy donors, with MRx0005 classified within the *phylum Bacteroidetes* and MRx0029 within the *phylum Firmicutes*, both of which represent dominant constituents of the human gut microbiota [238]. MRx0005 demonstrated the capacity to reduce levels of the pro-inflammatory cytokine IL-8 and inhibit nuclear factor kappa B subunit (NF-κB)-Ap1 promoter activation following LPS induction. In contrast, MRx0029 elevated IL-8 levels and activated the NF-κB-Ap1 promoter in the absence of induction. Both strains demonstrated antioxidant capacity, with MRx0029 exhibiting superior activity compared to MRx0005 [238]. MRx0029 also displayed a cell-type-dependent protective effect on undifferentiated SH-SY5Y cells against hydrogen peroxide (H_2O_2)-induced reactive oxygen species (ROS) cytotoxicity, which is implicated in neuroinflammation and neurodegeneration. MRx0029 not only modified the morphology of these cells by inducing a differentiated phenotype but also upregulated the expression of microtubule associated protein 2 (MAP2) and synaptophysin (SYP) [238]. Furthermore, MRx0029 predominantly produced butyric, valeric, and hexanoic acids, while MRx0005 primarily produced acetic and propanoic acids, further exhibiting their neuroprotective effects upon cellular treatment [238]. In addition, another study has evaluated the role of SCFAs in the immunomodulation of glioma [217]. Gut microbiota dysbiosis induced by antibiotic supplementation reduced the concentrations of propionic acid, butyric acid, valeric acid and caproic acid in the brain tumour tissues and serum compared to controls. In addition, the levels of these metabolites were positively correlated with the abundance of the *phylum Firmicutes* and *phylum Bacteroidetes* and negatively correlated with the *phylum Proteobacteria*, indicating that these microbiota might be the source of these

metabolites [217]. Besides, supplementation of SCFAs mixture including propionic acid, butyric acid, and valeric acid in drinking water to antibiotic-treated GBM mice improved the survival of mice and decreased the tumour volume. This supplementation restored the antibiotic treatment-induced polarisation of TAMs towards M1, characterised by an increased $CD86^+/CD206^+$ ratio and % of $CD86^+$ TAMs and decreased % of $CD206^+$ TAMs [217]. This SCFAs-induced M1 polarisation was driven by enhanced glycolysis in the TAMs characterised by the increased mRNA expression of hypoxia inducible factor 1 (HIF-1), glucose transporter 1 (GLUT1), hexokinase 2 (HK2), pyruvate kinase M1/2 (PKM2), and lactate dehydrogenase A (LDHA) along with an increased enzymatic activity of PKM2 and LDHA [217]. Further, SCFAs supplementation exhibited no anti-tumour effect in antibiotic-treated GBM mice lacking macrophages, suggesting that the SCFAs-mediated anti-tumour activity was dependent on macrophages [217].

Beyond maintaining host health, metabolites produced by specific microbial strains also influence the efficacy of therapeutic regimens in brain cancer. These bioactive compounds regulate the treatment outcomes by modulating the TME and immune responses. A study has demonstrated that temozolomide treatment induces metabolic adaptations in mice [193]. The alteration in the levels of gut microbial communities following temozolomide treatment may contribute to anti-tumour activity by influencing diverse pathways, including sesquiterpenoid and triterpenoid biosynthesis, steroid and terpenoids biosynthesis, fatty acid biosynthesis and metabolism, glutathione metabolism, mineral absorption, glucose and lipid metabolism, anti-inflammatory responses, immunomodulation, and epigenetic regulation through folate production [193, 239–246]. It has also been demonstrated that the upregulation of glutathione and fatty acid metabolism following temozolomide treatment might be associated with oxidative stress and fatty acid levels [193]. In another study, metabolomic analysis revealed the difference in levels of metabolites related to gut microbiota of mice bearing glioma cells sensitive and non-sensitive to temozolomide [192]. Further, pathway-based functional prediction from the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database revealed that steroid biosynthesis, sphingolipid metabolism and tryptophan metabolism were varied between the pre-dose faecal samples in the above-mentioned groups of mice. Notably, it has been reported that the gut microbes could metabolise tryptophan into tryptamine, kynurenine and indole derivatives involving the indoleamine 2,3-dioxygenase 1 (IDO1) and aryl hydrocarbon receptor (AHR) that have crucial roles in the immune homeostasis and tumour immune microenvironment respectively [247–251]. Also, tryptophan hydroxylase 1, a tryptophan metabolic enzyme, could promote glioma progression through the serotonin/L1 cell adhesion molecule (L1CAM)/NF-κB signalling pathway [250, 252]. Besides, the AHR and IDO1 mRNA expression were decreased in the brain and tumour tissues of mice bearing tumour cells that are non-sensitive to temozolomide [192]. In addition, it was also reported that the presence of distinct metabolites such as the Asn-Pro-Arg, deoxyuridine, DG(14:1/22:6), FFA(18:2), Glu-Met, Glycyl-Tryptophan, linolenic acid, hyroxyhexadecanoic acid, indole-3-lactic acid, isoleucine, Leu-Glu, MG(18:0), norvaline, oxindole, oxohexadecanoic acid, PA (8:0/13:0), PE (18:4), pentadecanoic acid, tryptophan, Tyr-Leu-Arg, and uridine was markedly associated with pharmacodynamic assessment indices such as total flux and tumour inhibition rate [192]. Interestingly, another study has reported that gut microbiota plays a crucial role in modulating the efficacy of Traditional Chinese Medicine [253]. It has been reported that coculturing blood serum from Taohong Siwu Decoction-treated SPF mice with glioma cells markedly suppressed the proliferation and clone-forming ability, compared to coculture with serum from untreated SPF mice and treated germ-free mice. In addition, pathway analysis has shown that DNA replication pathway was significantly modified, and differential gene analysis revealed that colony stimulating factor 3 (CSF3), plasminogen activator, urokinase (PLAU), ubiquitin like with PHD and ring finger domains 1 (UHRF1), FOS like 1, AP-1 transcription factor subunit (FOSL1), IL1B, dual specificity phosphatase 6 (DUSP6), minichromosome maintenance complex component (MCM)2, cyclin D1 (CCND1), Wnt family member 7B (WNT7B), MCM5, MCL1 apoptosis regulator (MCL1), SH2B adaptor protein 3 (SH2B3), MCM3, heterogeneous nuclear ribonucleoprotein M (HNRNPM), and paralemmin 2 and A-kinase anchoring protein 2 fusion (PALM2AKAP2) were upregulated and activating transcription factor 4 (ATF4), farnesyl-diphosphate farnesyltransferase 1 (FDFT1), adenylate cyclase 8 (ADCY8), cytochrome P450 family 1 subfamily B member 1 (CYP1B1), epiregulin (EREG), family with sequence similarity 114 member A1 (FAM114A1), eukaryotic translation initiation factor 4A2 (EIF4A2), aldo-keto reductase family 1 member B (AKR1B1), mitogen-activated protein kinase 1 interacting protein 1 like (C14orf132), collagen type I alpha 2 chain (COL1A2), cyclin dependent kinase inhibitor 1 A (CDKN1A), aspartate beta-hydroxylase (ASPH), DEPP autophagy regulator 1 (DEPP1), aldo-keto reductase family 1 member C1 (AKR1C1), and DNA damage inducible transcript 4 (DDIT4) were downregulated in treated SPF mice when compared to the untreated mice [253]. Additionally, cell division cycle 6 (CDC6) and minichromosome maintenance 10 replication initiation factor (MCM10), which are associated with the DNA replication pathway, were

also downregulated in treated SPF mice [253]. This study demonstrates that the metabolism of the gut microbiota plays a crucial role in the anti-tumour response against GBM. However, the specific target microorganisms and metabolites involved in this mechanism require further elucidation. Further, another study has elucidated the therapeutic potential of 20-O-β-(d-glucopyranosyl)-20(S)-protopanaxadiol (CK), a metabolite of oral ginseng produced by gut microbiota [254]. Administration of CK to C6 rat glioma cells reduced stromal cell-derived growth factor 1 (SDF-1) induced invasion and migration, characterised by the decreased expression of matrix metallopeptidase (MMP)9, MMP2, p-protein kinase C alpha (PKCα) and p-extracellular-signal-regulated kinase (ERK)1/2 [254].

These studies suggest that microbial metabolites exert regulatory effects on brain cancer progression through the GBA. The interplay between gut microbiota and the brain, mediated by various metabolites, may influence tumourigenic pathways in the brain. This relationship highlights the potential role of gut-derived factors in modulating the TME and may provide new avenues for therapeutic intervention. Further research is needed to elucidate the specific mechanisms through which microbial metabolites influence brain cancer development and progression via the GBA.

Clinical significance and applicability

It is now well established that the gut microbiota plays a crucial role in modulating the bidirectional communication between the CNS and the ENS. A plethora of studies have shown that manipulating the gut microbiome leads to amelioration of frontoparietal and subcortical brain activity with cognitive function in minimal hepatic encephalopathic patients [255]. The GBA, linking the gut microbiota and the CNS, could also be implicated in the development of brain cancer [256]. Microbiome are known to impact inflammation, immunity and response to treatments, which are vital factors in progression of cancer [257]. Several studies reviewed here have demonstrated an association between gut microbiota dysbiosis and glioma development, with microbial composition alterations varying based on treatment strategies. For example, a significant increase in alpha diversity was observed in GBM patients compared to healthy controls. At the phylum level, GBM cohorts exhibited a reduction in Firmicutes and a relative increase in Proteobacteria [256]. Similarly, in another study, it was reported that six different bacterial genera, including *Bifidobacterium*, *Bacteroides*, *Lachnospira*, *Fusobacterium*, *Parasutterella*, and *Escherichia/Shigella* could be used as microbial markers to distinguish brain tumours from healthy controls with an area under the curve (AUC) of 0.77 [183]. In addition, another study has developed a biomarker panel containing six abundant genera, including *Fusobacterium*, *Akkermansia*, *Escherichia/Shigella*, *Lachnospira*, *Agathobacter*, and *Bifidobacterium* with an AUC of 0.852 [182]. Moreover, another study demonstrated that extracellular vesicles of specific bacterial taxa could serve as potential biomarkers at different taxonomic levels [189]. At the phylum level, *Actinobacteria*, *Proteobacteria*, and *Firmicutes* were recognised as key indicators, while at the genus level, *Ruminococcaceae UCG-014*, *Turicibacter*, *Lactococcus*, and *Lactobacillus*, among other species, were suggested as potential biomarkers for the detection of brain tumours [189]. This suggests that gut microbiome analysis could potentially serve as a non-invasive diagnostic tool for detecting brain tumours. Preclinical studies have demonstrated that chemotherapy and immunotherapy induce alterations in gut microbiota composition, which may serve as valuable biomarkers for therapeutic monitoring and prognosis in gliomas [258]. Notably, understanding these microbiome changes is essential for elucidating tumour progression mechanisms, development of specific biomarker strategies and optimising therapeutic approaches, potentially contributing to improved clinical management of the disease. Microbial-derived metabolites such as butyrate, propionate, SCFAs, and acetate are known to modulate immune activation and response and, thereby, can be associated with cancer immunity [259]. Moreover, these metabolites can impact the BBB and enter the CNS, which could be vital when considering therapeutic efficacy [260, 261]. In another study, it was observed that certain metabolites such as norepinephrine and 5-hydroxy indole acetic acid were decreased in glioma patient faecal samples compared to controls [236]. Similarly, Herbreteau et al. observed a decline in SCFAs such as propionate and acetate, in the cecum of GL261 xenograft mice [196]. There are similar instances of decrease in SCFAs producing microbiota in the gut in glioma patients which could be employed to understand the glioma progression. These findings highlight the possible utility of SCFA alterations as biomarkers for disease monitoring and therapeutic interventions. Further, SCFAs are also known to play a crucial role in modulating immune responses by influencing both regulatory and effector T cell function through epigenetic and metabolic reprogramming [262]. Therefore, further research is warranted to investigate the therapeutic potential of SCFAs supplementation via microbiota modulation in combination with targeted therapies. Such studies could contribute to developing novel treatment strategies for brain cancer, enhancing therapeutic efficacy and patient outcomes.

Challenges or limitations

Understanding the role of gut microbiota composition in brain tumourigenesis may pave the way for alternate novel therapeutic strategies. Microbial flora in tumours exhibited to be discrete from those in non-tumoural tissues, either in terms of distinct microbial populations or variations in their abundance and can modulate various pathological conditions [187]. Detection of these differences in the composition of microbial flora remains invaluable in these instances. The utilisation of metagenomics within clinical settings for disease diagnosis has seen a marked increase, attributable to its capacity for culture-independent identification [263]. FDA has recommended the general guidelines for validation of the clinical NGS for testing infectious diseases [264]. However, implementation of these strategies in the clinical laboratory for pathogen detection includes many factors and elements, including less sensitivity with high background, laboratory workflow, reference standards, challenges in bioinformatic data analysis, cost, and regulatory considerations, that makes it to be practical [265]. The other challenges linked with the identification of the association of gut microbiota with brain cancer is to analyse the difference in the composition between tumour patients and healthy controls. Mendelian randomisation uses genetic variations to analyse the causal association between the gut microbial composition and brain cancer subtypes [169]. Even though the Mendelian randomisation is a powerful analysis, further animal experiments are required to unravel the molecular mechanisms and validate these findings. Besides, only a limited number of brain cancer subtypes, including glioma, GBM, meningioma, and pituitary adenoma, have been investigated for their association with gut microbiota as discussed above. Moreover, future studies should also investigate gut microbial composition across various stages of brain cancer patients, as this aspect remains largely unexplored. Further, the association between gut microbial composition and commonalities of brain cancer such as mutation frequencies, altered pathways, immune signatures should also be explored to progress towards devising better therapeutic strategies. To advance our understanding of the complex relationship between gut microbiota and brain cancer, it is imperative that we surmount these methodological obstacles.

One of the major limitations of clinical trials investigating the GBA is the relatively small sample sizes, which may not sufficiently capture the variability in gut microbiome composition among patients. Ethical considerations play a crucial role in the use of antibiotics, probiotics, and animal models in clinical trials. While these interventions hold therapeutic potential, concerns regarding patient safety, regulatory approvals, and the reproducibility of animal model findings must be carefully addressed. Following ethical considerations strictly is a good practice to ensure the responsible translation of microbiota-based therapies into clinical applications, maintaining both scientific integrity and patient welfare. Additionally, differences in study design, patient demographics, and treatment protocols contribute to inconsistencies in findings. The another critical factor that needs to be considered in clinical trials is the race and ethnicity of the patients included in the study population [266–268]. Considering the impact of geographical factors and ethnic background on gut microbiota composition, findings from trials involving specific ethnic groups may not necessarily translate to benefits for other ethnic populations [75, 269]. To overcome these challenges, future research should prioritize large-scale, multicentre, and multifactorial randomised clinical trials. Such studies will improve the reliability and generalisability of findings, facilitating a deeper understanding of gut microbiome alterations in disease progression and therapeutic response. Ultimately, these efforts will be crucial in developing personalised treatment strategies tailored to diverse patient populations.

Future perspectives

Microbiota-based therapeutic strategies for GBM: probiotics, FMT, and dietary interventions

The dysbiosis of gut microbiota composition that occurred after tumourigenesis can be ameliorated by the treatment with probiotics. Accumulating shreds of evidence have examined the beneficial effects of probiotics on human health [15–17, 19]. Apart from this, research over the years has also reported the complex interplay of probiotics and their metabolites in the modulation of PI3K/Akt/mechanistic target of rapamycin kinase (mTOR) signalling pathways, which is one of the dysregulated pathways in various cancers, including brain cancers [49, 270, 271]. Employing probiotics for the treatment of brain cancer would be an improved alternative therapeutic regimen. For instance, mice bearing GL261 tumour cells treated with *Bifidobacterium lactis* and *Lactobacillus plantarum* via oral gavage reduced the tumour volume, and number of $Ki67^+$ cells and improved the survival time [272]. In addition, this treatment improved the neurobehavior of mice characterised by the decreased clinical and beam balance test score and ameliorated intestinal barrier damage by increasing Occludin protein expression. Further, it modulated the PI3K/Akt pathway by downregulating p-PI3K and Survivin expression while upregulating the PTEN expression [272]. Probiotic treatment increased the abundance of *Firmicutes* and decreased *Bacteroidetes* and also increased the level of metabolites such as threonic acid, conduritol b epoxide 1, and ascorbate and decreased the level of D-arabitol and elaidic acid compared to the control group [272].

Moreover, a comparable study has investigated the therapeutic potential of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* in modulating glioma tumour progression [273]. Administration of these bacterial strains in a glioma mice model has elicited a significant reduction in tumour volume while prolonging survival, without inducing hepatic or renal toxicity. However, it failed to mitigate glioma-induced disruptions in intestinal barrier integrity [273]. This study has also shown that *Bifidobacterium* strains administration has altered the composition of tumour microbiota characterised by the increased Chao1, Shannon, and Simpson indices. At the phylum level, the cohort receiving bacterial administration demonstrated an elevated relative abundance of *Firmicutes*, *Bacteroidetes*, *Cyanobacteria*, and *Actinobacteria*, concomitant with a decreased prevalence of *Proteobacteria* in comparison to the control group [273]. At the genus level, the administered group exhibited a significantly higher abundance of *Enterobacter*, *Citrobacter*, *Acinetobacter*, *Wautersiella*, *Enhydrobacter*, and *Escherichia/Shigella* relative to the control group. In addition, it was found that *Bifidobacterium* demonstrated increased abundance in both gut and tumour tissues modulating various serum metabolites involved in the modulation of important signalling pathways that regulate the tumour progression [273]. Notably, administering these bacterial strains attenuated tumour progression by suppressing the mitogen-activated protein kinase kinase (MEK)/ERK signalling cascade, which is predominantly implicated in the invasive phenotype of glioma cells. This effect was characterised by a marked reduction in phosphorylated MEK and ERK1/2 levels, alongside the downregulation of Wnt5a mRNA expression [273]. Another study has demonstrated the cytotoxic effect of kefir drink on the GBM cell line U87 [274]. Further, probiotics have been enacted in clinical trials involving patients of other diseases [275–278]. These studies demonstrate the therapeutic potential of probiotics in the treatment of brain cancer and its subtypes.

FMT involves the transfer of stool from a healthy donor into the gastrointestinal tract of a recipient to restore a balanced gut microbiome [279–281]. It has demonstrated high efficacy in treating recurrent *Clostridium difficile* infections unresponsive to standard antibiotic therapies [281]. Beyond gastrointestinal disorders, FMT is being explored as a potential therapeutic approach for extra-intestinal conditions, including metabolic and neurological disorders, due to its capacity to modulate gut microbiota composition [279–281]. FMT ameliorates the tumour progression in brain cancer by restoring gut microbial diversity and modulating systemic immune responses. This intervention enhances anti-tumour immunity by influencing the GBA, leading to alterations in immune cell infiltration, cytokine signalling, and metabolic pathways within the TME [282]. FMT from a healthy donor ameliorates the tumour progression, whereas from a diseased individual exacerbates the tumour progression. For instance, the induction of gut dysbiosis with antibiotics in the glioma mice model promoted tumour growth [194]. Restoration of a balanced gut microbiome with FMT from normal mice has reduced tumour growth by upregulating the expression of FOXP3 [194]. Another study has reported that the FMT in antibiotic-treated mice has reduced the tumour volume and significantly improved the survival of the GBM mice model [217]. Conversely, FMT from $Nf1^{OPG}$ tumour mice to conventionally raised germ-free mice has worsened the tumour progression, characterised by increased $Ki67^{+}$ and $Blbp^{+}$ cells [283]. Findings from these in vivo studies suggest that FMT holds significant potential as a therapeutic strategy for brain cancer by modulating the GBA and influencing the TME. However, further comprehensive investigations are required to validate these results, including mechanistic studies to elucidate the underlying pathways.

Dietary interventions are crucial in modulating the gut microbiota, influencing cancer development and progression [284, 285]. Specific dietary components, such as fibre, polyphenols, and omega-3 fatty acids, promote the growth of beneficial microbes, producing SCFAs with anti-inflammatory and anti-tumour properties [241, 137, 286]. Conversely, high-fat and high-protein diets can induce dysbiosis, fostering a pro-tumourigenic microenvironment through microbial metabolites such as secondary bile acids and trimethylamine-N-oxide (TMAO) [287, 288]. Emerging evidence suggests that gut microbiota-mediated dietary modulation can enhance therapeutic outcomes in cancer. For instance, a study has evaluated the impact of dietary component addition on brain cancer risk using a machine learning model [189]. This experiment demonstrated that incorporating sorghum, brown rice, oil, garlic, fermented beans, mealworm, turmeric, cabbage, shiitake mushroom, and onion into the diet of high-fat diet-fed mice reduced brain tumour risk, whereas the inclusion of pear and bellflower increased the risk [189]. It has also been reported that including fermented beans reduced brain tumour risk, whereas roasted beans increased the risk, highlighting the significant influence of food processing methods on brain cancer risk [189]. Another study has reported that the consumption of RO water supplemented with SCFAs such as propionic acid, butyric acid, and valeric acid to the antibiotic-treated mice restored gut microbial homeostasis in antibiotic-treated mice [217]. This intervention exerted an anti-tumour effect by inducing metabolic reprogramming in macrophages, specifically enhancing glycolysis and facilitating polarisation towards the M1 phenotype [217]. Moreover, another study has

demonstrated enhanced survival rates in a GBM mouse model upon supplementing a high glucose drink, suggesting its potential therapeutic benefit [195]. This effect is attributed to the modulation of anti-tumour immunity, facilitated through alterations in the gut microbiota composition. Notably, high glucose drink administration promoted the colonisation of the *Desulfovibrionaceae* family in GBM mice, indicating a possible link between specific microbial populations and tumour progression [195]. These studies imply that dietary intervention plays a crucial role in brain cancer as a therapeutic regimen.

Further comprehensive investigations are required to validate these results, including mechanistic studies to elucidate the underlying pathways. Additionally, well-designed clinical trials are essential to assess the efficacy, safety, and translational potential of probiotics, FMT, and dietary interventions in brain cancer treatment. Rigorous clinical studies are essential to determine optimal dosages, treatment regimens, and long-term outcomes in diverse patient populations with brain cancer and its subtypes. These clinical trials also need to validate and expand the application of these regimens as a therapeutic approach, paving the way for their integration into clinical practice.

Artificial intelligence and bioinformatics in microbiome research and brain cancer therapy

For several decades, contemporary medicine has prioritised identifying disease-specific diagnostic, preventive, and therapeutic strategies. While many of these approaches have demonstrated efficacy in certain patients, their effectiveness has not been universal, as the individual-specific factors influencing disease manifestation and treatment response have largely remained unclear [289]. The variability among individuals, driven by environmental and genetic factors, poses a significant challenge in developing effective regimens of population-based early diagnostic, prognostic, and therapeutic assessments [290]. The human microbiome, comprising trillions of microbial inhabitants and diverse microbial communities, including viruses, bacteria, fungi, and eukaryotes, colonises human body surfaces and orchestrates an intricate symphony of physiological processes, significantly influencing health and disease states. It has also been identified as a contributing factor to variations among individuals through its unique, individual-specific signatures [290]. The likelihood of personalised medical therapies has accelerated therapeutic and diagnostic advancements that include detailed patient profiles, including demographics, family history, traditional laboratory data and next-generation omics data such as genomic, transcriptomic, metabolomic and proteomic datasets [291]. The emergence of high-throughput technologies offers unrivalled opportunities to investigate molecular mechanisms in health and disease conditions, however, it also presents challenges due to the high dimensionality and collinearity among features [292, 293]. Conventional statistical methods often prove inadequate in handling high-dimensional data, highlighting the need for innovative computational approaches capable of fully leveraging big data in bioinformatics [292, 294–296]. Artificial intelligence (AI) and bioinformatics have revolutionised biomedical research, offering unparalleled capabilities in data analysis, predictive modelling and personalised medicine. AI algorithms, especially machine learning, deep learning and neural networks, have emerged as indispensable tools for taxonomic profiling, functional annotation, and predictive modelling of microbial communities [297, 298].

The cancer characterisation of the microbiome data involves several steps such as sample collection, sequencing, taxa assignment and abundance calculation, decontamination, batch effects removal, feature selection, model training and validation and inference, as discussed elsewhere [299]. This section of the article highlights and discusses studies investigating the relationship between gut microbiota and brain cancer using AI and other computational algorithms. By examining their methodologies and findings, this article aims to provide a clear understanding of how these algorithms are applied in this area of research. Microbiome studies normally employ 16 S rRNA gene-targeted sequencing, whole metagenome shotgun sequencing, or other omics technologies to characterise its composition, taxonomy and dynamics [300]. The processing, analysis, and interpretation of these data require a range of computational tools designed to filter, cluster, annotate, and quantify the obtained data [300]. The commonly used methods for analysing the microbiome profiles at the genus level are OTUs and the amplicon sequence variants [182–184, 192, 193, 217, 253, 299, 301]. Microbiome abundance data used in cancer research presents a challenge for machine learning models due to its high dimensionality, sparsity, and compositional nature [297–299]. Apart from taxonomic profiles, the inclusion of functional microbiome data such as genes, proteins and metabolites and other microbiome data such as SNPs can enhance the machine learning models' performance [302–305]. For instance, in neuroblastoma, a brain cancer subtype, Li, Xin et al. have utilised a machine learning-mediated algorithm to evaluate the prognosis of the patients with microbial gene abundance score [304]. This group has utilised various algorithms such as Skmer for dissimilarity among samples and random forest survival analysis with microbial gene abundance score as input for predicting survival. Based on the microbial gene abundance score, the patients were stratified into different clusters and subsets exhibiting differential risk levels [304]. It has been reported that the

difference in survival probabilities of these patients was influenced by the expression of cAMP responsive element binding protein (CREB) and its target genes, such as BCL2 apoptosis regulator (Bcl-2), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and IGF-2. Also, the low survival probability in the patients might be due to the increased activation of CREB, which further promotes tumour progression [304]. To achieve high accuracy in machine learning models, preprocessing steps such as transformation, normalisation, and feature selection of microbial data should be performed before providing it as input to the models [299]. The feature transformation step in preprocessing may involve methods such as log transformation, cube-root normalisation, rescaling to a unitary scale, or the application of Bayesian models (HARMONIES) [299]. Dimensionality reduction in microbiome data analysis involves two primary approaches: feature extraction and feature selection. Feature extraction methods, such as Principal Component Analysis (PCA) and Principal Coordinates Analysis (PCoA), transform high-dimensional data into a reduced feature set [299]. Feature selection, on the other hand, identifies the most relevant features using statistical tests (e.g., t-test for binary classification, Mann-Whitney U test as a non-parametric alternative, and ANOVA for multiclass classification), Minimum Redundancy - Maximum Relevance (mRMR) for selecting uncorrelated and informative features, and Linear Discriminant Analysis Effect Size (LEfSe) for incorporating biological relevance [299]. Additionally, tree-based models, including random forest and Gradient-Boosted Trees, assign feature importance scores, while wrapper and embedded methods, such as Least Absolute Shrinkage and Selection Operator (LASSO) regression and Recursive Feature Elimination with Support Vector Machines (SVM-RFE), further refine feature selection through model-dependent approaches [299]. After the preprocessing, the data would be applied to models for the identification of the cancer-related microbiome [299]. The generally used machine learning models are support vector machines, decision tree-based methods such as random forests and boosting, logistic regression, artificial neural networks etc [299]. For instance, a study has developed a brain tumour diagnostic model and assessed the risk associated with consuming specific dietary components based on microbiome-derived extracellular vesicle data [189]. They have employed VSEARCH, a clustering method, UCLUST, Quantitative Insights Into Microbial Ecology (QIIME) and the Silva 132 database for assigning OTUs at the genus and species level [189]. The predictive diagnostic model was developed using the relative abundance of microbes at the genus levels as model variables, which was the result of four methods, stepwise selection and LEfSe algorithm either in combination with or without age and gender as covariates [189]. The stepwise and LEfSe algorithms yielded 12 and 29 significant microbial genera at the genus level, respectively, and the model developed with these algorithms has returned an AUC greater than 0.93. Another model, developed using the gradient boosting algorithm with microbial extracellular vesicle-analysed data as input, has exhibited the highest specificity, sensitivity, and AUC, achieving values of 1.000, 0.936, and 0.993, respectively [189]. LEfSe analysis of serum extracellular vesicle and tissue microbiome revealed a total of 30 genera with *Ruminococcaceae UCG-014* (LDA score > 4.0) and *Bacteroidales S24-7 group*, *Lachnospiraceae NK4A136*, *Bacteroides*, and *Erysipelatoclostridium* as significant biomarkers in the brain tumours respectively [189]. In addition, the impact of diet on the extracellular vesicle microbiome in relation to brain tumour risk was evaluated by incorporating diet-induced changes in genus-level relative abundances in high-fat diet-fed mice into stepwise selection and LEfSe models [189]. This study suggests that gut microbiota regulate human health through circulating extracellular vesicles and that these vesicles could serve as potent biomarkers for the assessment of brain cancer.

Numerous studies discussed in this article have utilised these computational algorithms to explore the intricate relationship between gut microbiota and brain cancer, including its various subtypes. These investigations have primarily focused on identifying microbial community differences and their potential roles in disease progression. Among the various analytical techniques employed, PCoA has emerged as the most widely used feature extraction method for differentiating microbial profiles across distinct sample groups [182, 183, 193, 195, 207, 306]. This method is frequently coupled with statistical tools such as non-metric multidimensional scaling (NMDS) and analysis of similarities (Anosim), which provides an additional dimension of data visualisation, enabling a more comprehensive understanding of microbial diversity and clustering patterns [182, 183, 193, 207, 283, 306]. In addition, to assess significant differences in microbial abundance among various experimental groups, the LEfSe algorithm has been extensively applied [182, 183, 306, 307]. This robust analytical tool identifies differences in microbial abundance among various experimental groups, facilitating a deeper insight into potential microbial biomarkers associated with brain cancer risk and progression [307]. Besides, the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), a bioinformatics tool that utilises reference genome databases and phylogenetic information, was employed to detect the functional profiling of these altered microbes using various databases such as the Greengene database and KEGG, and Cluster of Orthologous Groups for biochemical pathway-based

information [308]. Microbiome studies have widely applied this method to predict pathways involved in host health and disease, including brain cancer, by linking microbial composition to functional attributes. PICRUSt has proven particularly useful in exploring the GBA, as it identifies microbial functions that may influence neuroinflammatory and oncogenic processes [182, 183, 192, 193]. Moreover, QIIME2, a widely utilised bioinformatics tool, has also been employed to perform various functions, including sequence quality control, taxonomic classification, phylogenetic reconstruction, and diversity analyses, in the investigation of gut microbiota's role in brain cancer [182, 183, 192, 273, 309]. This open-source software enables comprehensive end-to-end analysis of diverse microbiome data, facilitating reproducible, scalable and interactive analyses and integrating a wide range of statistical tools [310, 311]. QIIME2 incorporates a decentralised provenance tracking system, which automatically logs each analysis step, ensuring full reproducibility and transparency in microbiome data science [310, 311]. These studies suggest that AI and bioinformatics algorithms have played a crucial role in advancing the investigation of gut microbiota in brain cancer and its subtypes. Integrating these computational approaches has facilitated the identification of microbial signatures, functional pathways, and potential biomarkers associated with disease onset and progression. AI-driven models, coupled with sophisticated bioinformatics pipelines, have enhanced the accuracy and efficiency of microbiome data analysis, enabling more profound insights into the complex interactions between gut microbiota and brain tumour development. Future research should further explore and refine these analytical tools, focusing on developing predictive models that can accurately assess the role of gut microbiota in brain cancer progression. Such advancements can potentially contribute to personalised therapeutic strategies and improve diagnostic capabilities in neuro-oncology.

Conclusion

The intricate system of molecular interactions has not only revolutionised our understanding of cellular communication but has also emphasised the profound symbiotic relationship between organisms. The symbiotic relationship between eukaryotic cells and bacteria is a longstanding practice dating back to ancient times. The remarkable exchange of molecules serving as messengers and signals through signalling pathways stands as a pivotal cornerstone in the biological landscape. The human body harbours a diverse and complex community of microbes residing or colonised at different sites. This microbiota offers numerous benefits to the host through a range of various physiological and pathological processes. The GBA is the bridge that connects the gut microbial composition and brain health regulation. Notably, brain tumourigenesis may cause dysbiosis of the gut microbiota, as evidenced in mice model studies discussed above. Clinical and randomisation studies have shown remarkable structural and functional changes in gut microbial composition in tumour patients compared to controls. The diverse array of microbiota residing in the gut exerts a significant regulatory influence on brain tumour cells. This consortium of bacterial genera orchestrates the modulation of the immune system, facilitating communication via an intricate network of metabolites and cytokines. Such interactions can lead to the targeted eradication of tumour cells, emphasising the microbiota's pivotal role in the body's defence against neoplastic diseases within the brain. This complex interplay highlights the potential for leveraging gut microbiota in developing innovative treatments for brain tumours and their subtypes. Fascinatingly, the presence of specific microbes plays a crucial role in conciliating the sensitivity and efficacy of chemotherapeutic drugs. These microorganisms can either enhance or diminish the effectiveness of cancer treatments, depending on their interaction with the drugs. Identifying and understanding these microbial species might offer a promising alternative approach to overcome therapeutic resistance. In addition, the above-discussed studies indicate that specific microbial strains, such as *Desulfovibrio vulgaris*, *Bifidobacterium lactis*, *Lactobacillus plantarum*, *B. breve*, *B. longum*, and *B. bifidum*, exhibit anti-tumourigenic effects, whereas *Bacteroides* demonstrate tumour-promoting properties in brain cancer and its subtypes. Notably, microbial strains with anti-tumour potential are downregulated in brain cancer patients, suggesting their potential as biomarkers for disease detection and prognosis. Further research is warranted to validate these findings, and future clinical trials should consider these strains as therapeutic candidates, assessing their clinical safety, optimal dosage, and efficacy in brain cancer treatment. By targeting or administering these microbes, we may improve the success rates of therapies and provide more effective treatment options for brain cancer patients. As we continue to unravel the complexities of molecular communication, it becomes increasingly evident that this reciprocal exchange is not merely advantageous but essential for the well-being and sustainability of both mammalian cells and bacteria alike.

Abbreviations

5-HT	5-hydroxytryptamine
ADCY8	Adenylate cyclase 8
AHR	Aryl hydrocarbon receptor
AI	Artificial intelligence
AKR1B1	Aldo-keto reductase family 1 member B
AKR1C1	Aldo-keto reductase family 1 member C1
Akt	Protein kinase B
Anosim	Analysis of similarities

ANS	Autonomic nervous system
ARG1	Arginase 1
ASPH	Aspartate beta-hydroxylase
ATF4	Activating transcription factor 4
AUC	Area under the curve
BBB	Blood-brain barrier
Bcl-2	BCL2 apoptosis regulator
BDNF	Brain-derived neurotrophic factor
CD8α	Cytotoxic T lymphocytes
CDC6	Cell division cycle 6
CDKN1A	Cyclin dependent kinase inhibitor 1 A
CK	20-O-β-(d-glucopyranosyl)-20(S)-protopanaxadiol
CNS	Central nervous system
COL1A2	Collagen type I alpha 2 chain
CRC	Colorectal cancer
CREB	cAMP responsive element binding protein
CRUK	Cancer Research UK
CSF3	Colony stimulating factor 3
CSF	Cerebrospinal fluid
CTLA4	Cytotoxic T-lymphocyte associated protein 4
CYP1B1	Cytochrome P450 family 1 subfamily B member 1
DDIT4	DNA damage inducible transcript 4
DEPP1	DEPP autophagy regulator 1
DUSP6	Dual specificity phosphatase 6
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
EIF4A2	Eukaryotic translation initiation factor 4A2
ENS	Enteric nervous system
EREG	Epiregulin
ERK	Extracellular signal regulated kinase
FAM114A1	Family with sequence similarity 114 member A1
FDA	Food and Drug Administration
FDFT1	Farnesyl-diphosphate farnesyltransferase 1
FMT	Faecal microbiota transplantation
FOSL1	FOS like 1, AP-1 transcription factor subunit
FOXP3	Forkhead box P3
FXR	Farnesoid X receptor
GABA	Gamma-aminobutyric acid
GBA	Gut-brain axis
GBM	Glioblastoma
GH	Growth hormone
GLUT1	Glucose transporter 1
GWAS	Genome-wide association studies
H_2O_2	Hydrogen peroxide
HDAC	Histone deacetylase
HIF-1	Hypoxia inducible factor 1
HK2	Hexokinase 2
HNRNPM	Heterogeneous nuclear ribonucleoprotein M
HPA	Hypothalamic pituitary adrenal
IDH	Iso-citrate dehydrogenase (NADP(+))
IDO1	Indoleamine 2,3-dioxygenase 1
Ifi	Interferon alpha inducible protein
Ifit	Interferon-induced protein with tetratricopeptide repeats
Ifitm	Interferon induced transmembrane protein
IFN-γ	Interferon gamma
IGF-1	Insulin like growth factor 1
IGF	Insulin-like growth factor
IL	Interleukin
iNOS	Inducible nitric oxide synthase
Isg	Interferon-stimulated genes
JAK	Janus kinase
KEGG	Kyoto Encyclopaedia of Genes and Genomes
L1CAM	L1 cell adhesion molecule
LASSO	Least absolute shrinkage and selection operator
LDHA	Lactate dehydrogenase A
LEfSe	Linear discriminant analysis effect size
LPS	Lipopolysaccharide
MAP2	Microtubule associated protein 2
MCL1	MCL1 apoptosis regulator
MCM10	Minichromosome maintenance 10 replication initiation factor
MCM	Minichromosome maintenance complex component
CCND1	Cyclin D1
MEK	Mitogen-activated protein kinase kinase
MGMT	O-6-methylguanine-DNA methyltransferase
MMP	Matrix metallopeptidase
mRMR	Minimum redundancy-maximum relevance
mTOR	Mechanistic target of rapamycin kinase
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NMDS	Non-metric multidimensional scaling
NOG	NOD/Shi-SCID, IL-2Rγnull
NTRK	Neurotrophic tyrosine receptor kinase
OTU	Operational taxonomic units
P2RY12	Purinergic receptor P2Y12
PALM2AKAP2	Paralemmin 2 and A-kinase anchoring protein 2 fusion
PCA	Principal component analysis
PCoA	Principal coordinates analysis
PD-1	Programmed cell death 1
PD-L1	Programmed cell death 1 ligand 1
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
PKCα	Protein kinase C alpha
PKM2	Pyruvate kinase M1/2
PLAU	Plasminogen activator, urokinase
PNS	Peripheral nervous system
PTEN	Phosphatase and tensin homolog
RAS	Rat sarcoma
ROS	Reactive oxygen species
RTK	Receptor tyrosine kinase
SCFA	Short-chain fatty acids
SCN	Suprachiasmatic nucleus
SH2B3	SH2B adaptor protein 3
STAT	Signal transducer and activator of transcription
SVM-RFE	Recursive Feature Elimination with Support Vector Machines
T_c cell	Cytotoxic T cell
T_{reg}	Regulatory T cell
TAMs	Tumour associated macrophages
TCGA	The Cancer Genome Atlas
TGFβ	Transforming growth factor beta
TGR5	Takeda G-protein-coupled receptor 5
TLR	Toll-like receptor
TMAO	Trimethylamine-N-oxide
TME	Tumour microenvironment
TNF	Tumor necrosis factor
TP53	Tumor protein p53
UHRF1	Ubiquitin like with PHD and ring finger domains 1
VEGF	Vascular endothelial growth factor
WHO	World Health Organisation
WNT7B	Wnt family member 7B
Wnt	Wingless-type MMTV integration site family
WT	Wildtype [312]

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